Fig. 1

Mutant-specific oligonucleotide primers used for mutant number 1. Mutated nucleotide underlined.

Bet v 1 sense	5'-	AATTATGAGACTGAGACCACCTCTGTTATCCCAGCAGCTCG –3'
Bet v 1 non-sense	3'-	TTAATACTCTGACTCTGGTGGAGACAATAGGGTCGTCGAGC –5'
sense primer	5'-	TGAGACCCCCTCTGTTATCCCAG –3'
non-sense primer	3'-	ATACTCTGACTCTGGGGGAGACA -5'

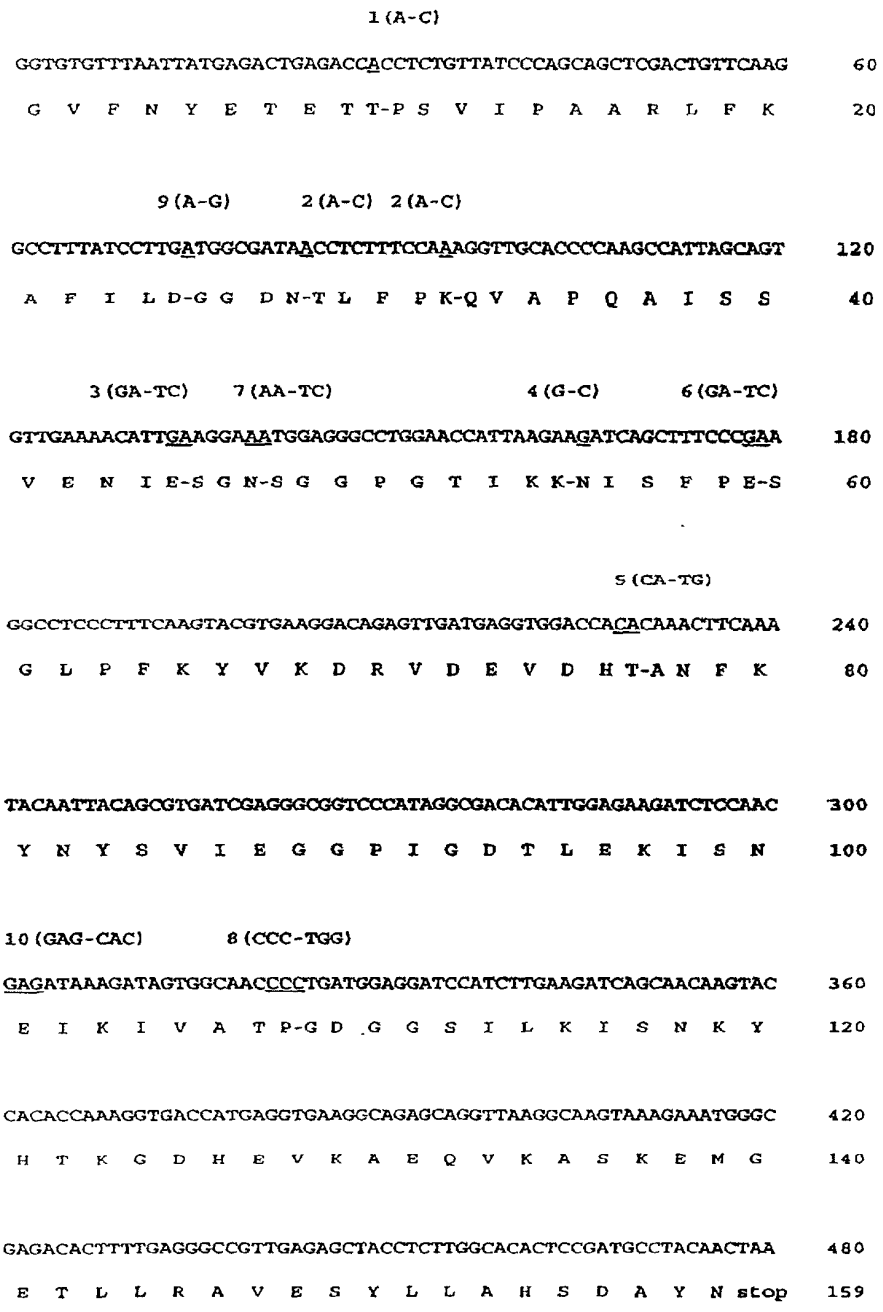
Fig. 2

Oligonucleotide primers for site directed mutagenesis of Bet v 1 (No. 2801).

all	sense	1: 183Bv, 15-mer 5'-GTTGCCAACGATCAG
1	sense	2: 184Bv, 23-mer 5'-TGAGACCCCCTCTGTTATCCCAG
1	non-sense	3: 185Bv, 23-mer 5'-ACAGAGGGGGTCTCAGTCTCATA
2	sense	4:186Bv, 31-mer 5'-GATACCCTCTTTCCACAGGTTGCACCCCAAG
2	non-sense	5: 187Bv, 31-mer 5'-ACCTGTGGAAAGAGGGTATCGCCATCAAGGA
3	sense	6: 188Bv, 23-mer 5'-AACATTTCAGGAAATGGAGGGCC
3	non-sense	7: 189Bv, 23-mer 5'-TTTCCTGAAATGTTTTCAACACT
4	sense	8: 190Bv, 23-mer 5'-TTAAGAACATCAGCTTTCCCGAA
4	non-sense	9: 191Bv, 23-mer 5'-AGCTGATGTTCTTAATGGTTCCA
5	sense	10: 192Bv, 23-mer 5'-GGACCATGCAAACTTCAAATACA
5	non-sense	11: 193Bv, 23-mer 5'-AGTTTGCATGGTCCACCTCATCA
6	sense	12: 194Bv, 23-mer 5'-TTTCCCTCAGGCCTCCCTTTCAA
6	non-sense	13: 195Bv, 23-mer 5'-AGGCCTGAGGGAAAGCTGATCTT
7	sense	14: 196Bv, 24-mer 5'-TGAAGGATCTGGAGGGCCTGGAAC
7	non-sense	15: 197Bv, 24-mer 5'-CCCTCCAGATCCTTCAATGTTTTC
8	sense	16: 198Bv, 24-mer 5'-GGCAACTGGTGATGGAGGATCCAT
8	non-sense	17: 199Bv, 24-mer 5'-CCATCACCAGTTGCCACTATCTTT
all	non-sense	18: 200Bv, 15-mer 5'-CATGCCATCCGTAAG

Fig. 3

Overview of all Bet v 1 mutations



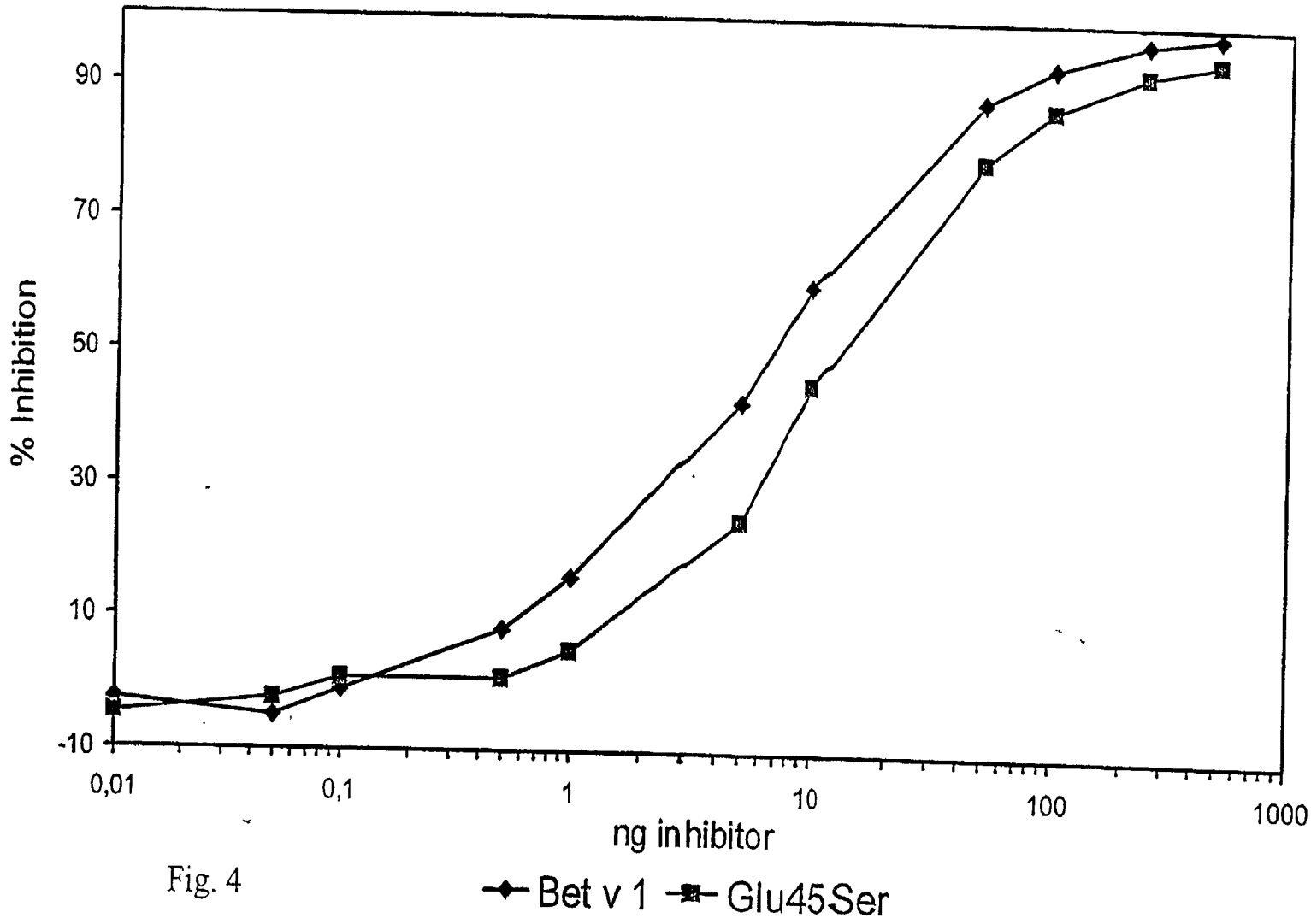


Fig. 4

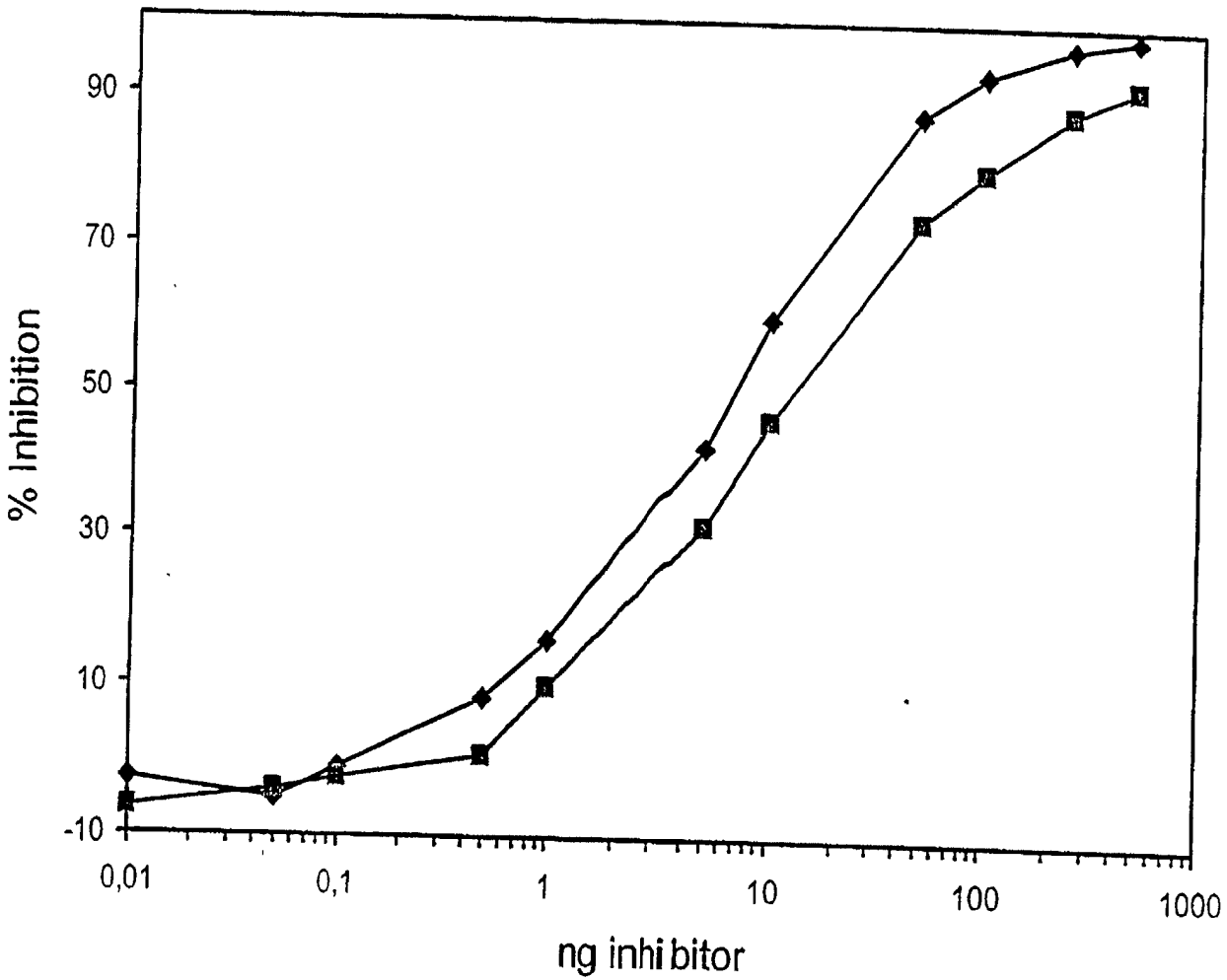


Fig. 5 —◆— Bet v 1 —■— Asn28Thr+Lys32Gln

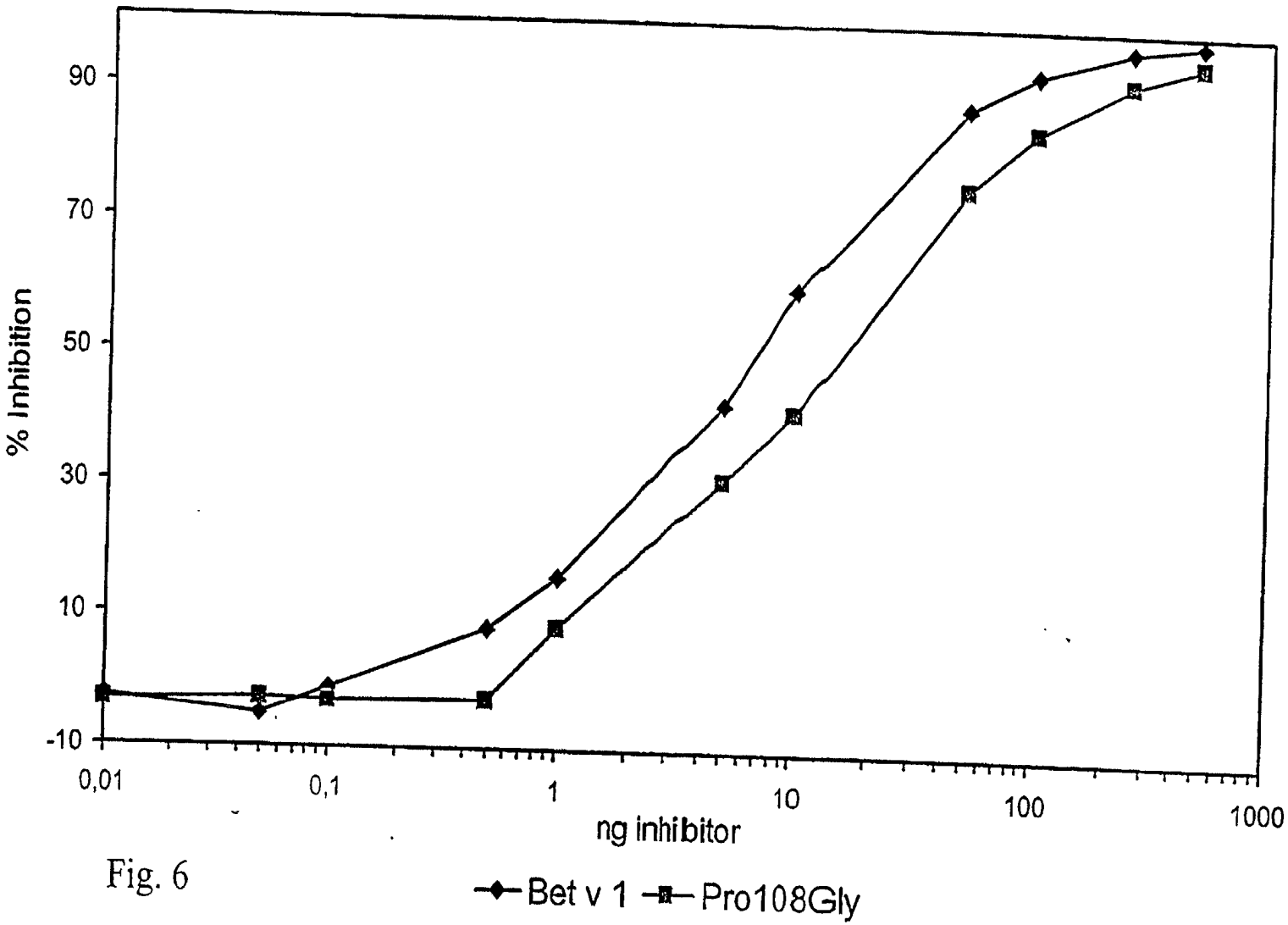

90
70
50
30
10
-10
% Inhibition
0,01
0,1
1
10
100
1000
ng inhibitor
Bet v 1
Pro108Gly

Fig. 6

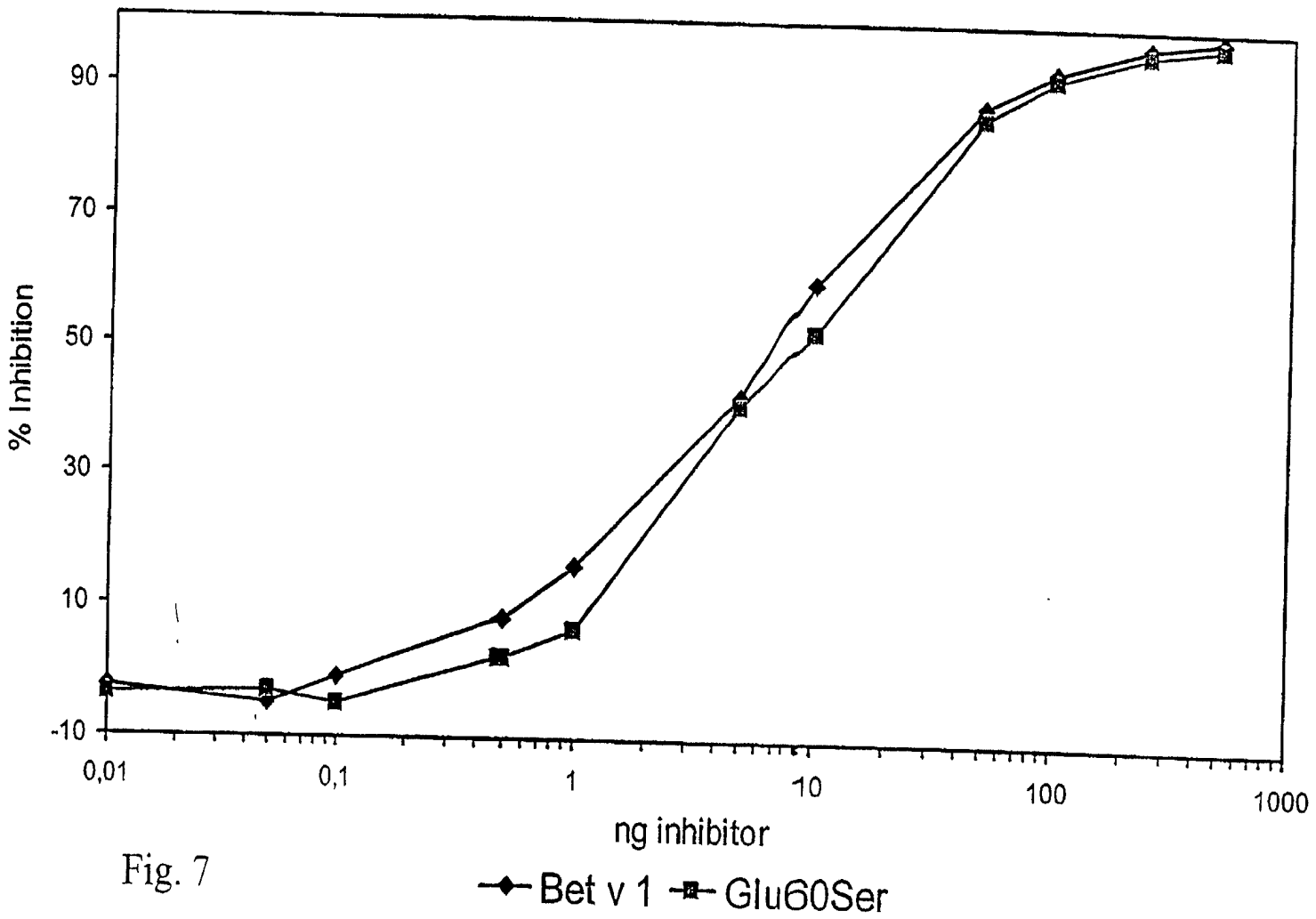


Fig. 7

10mM Na2HP04/NaH2P04 0, 02%Na3N3

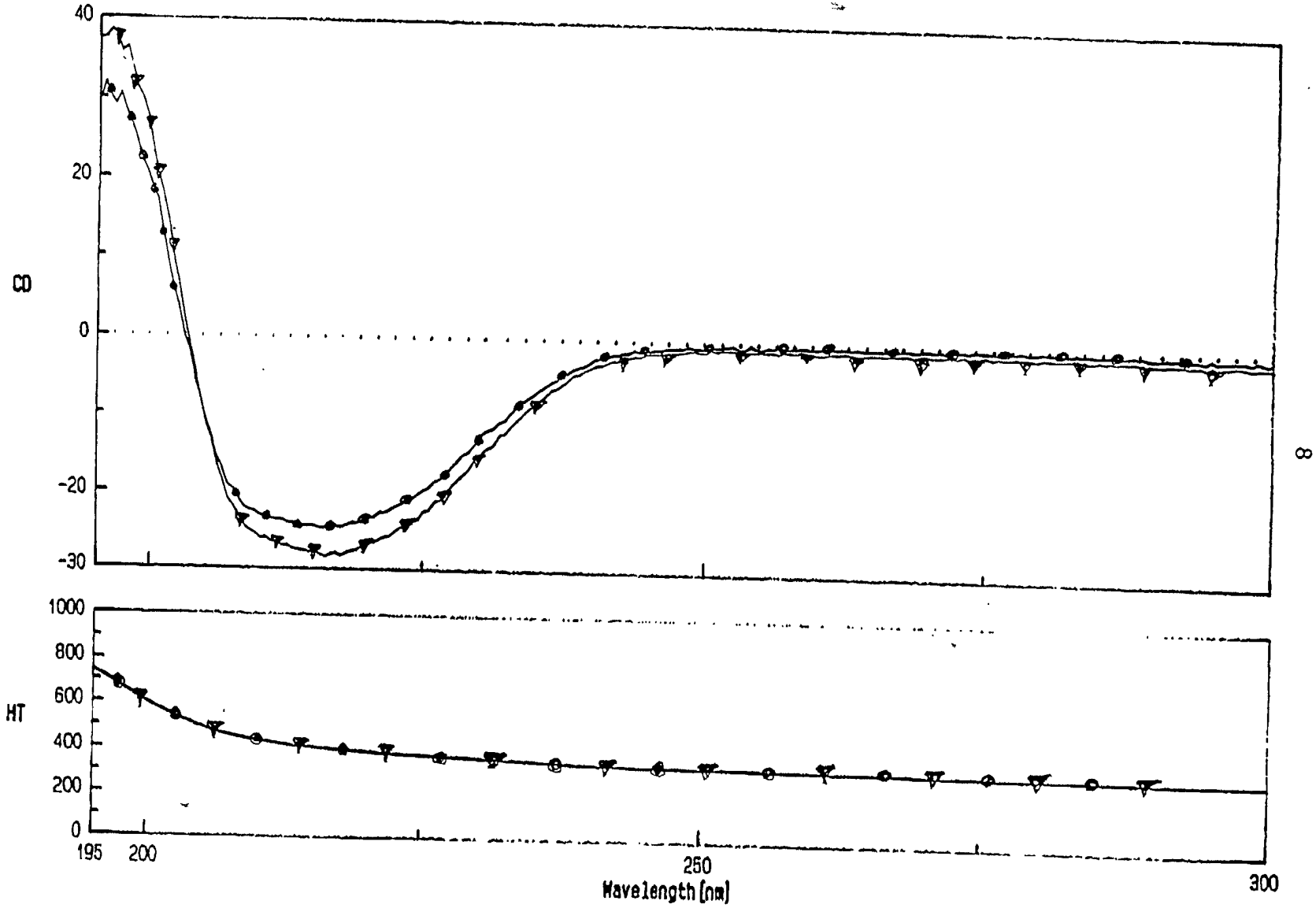


FIG. 8

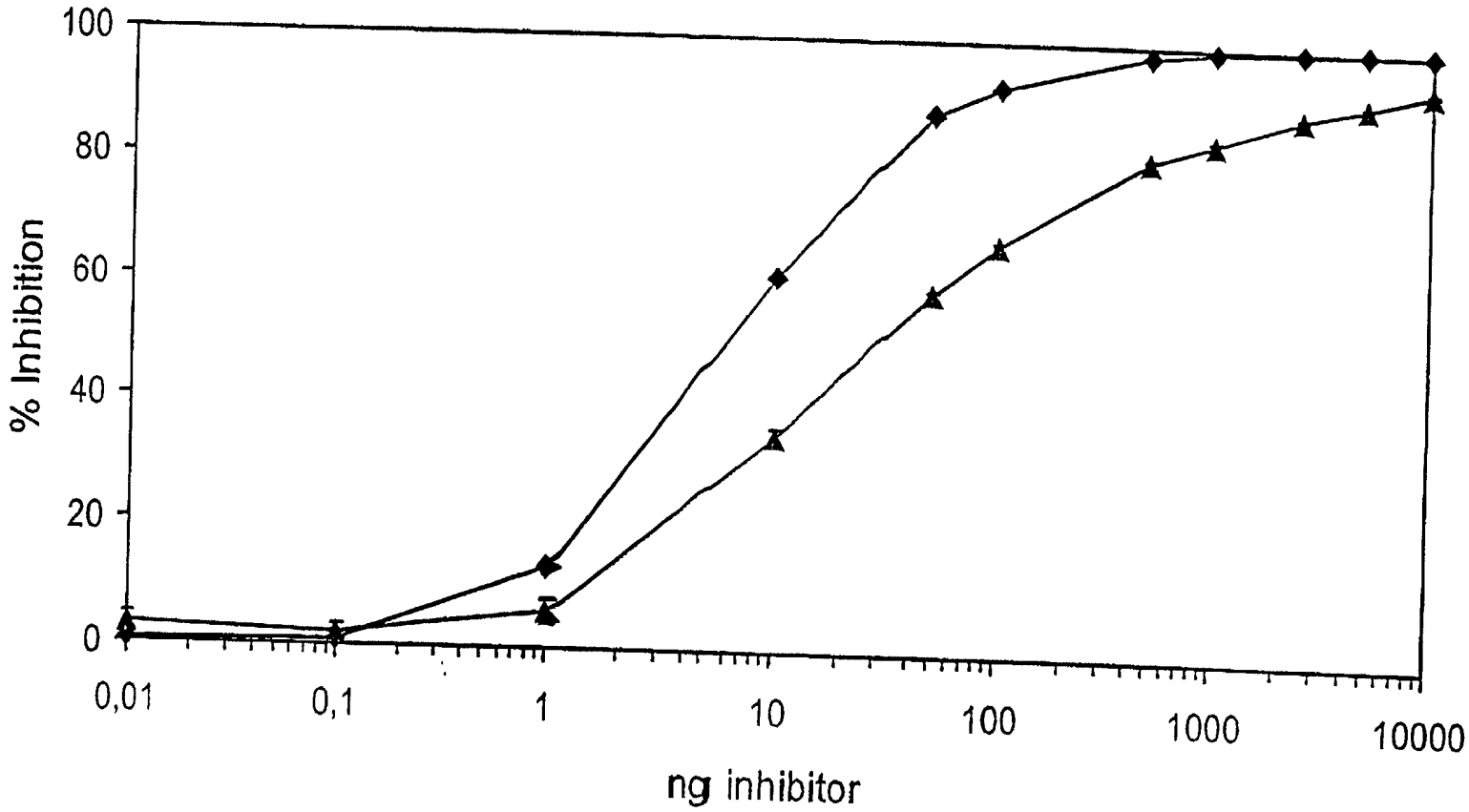
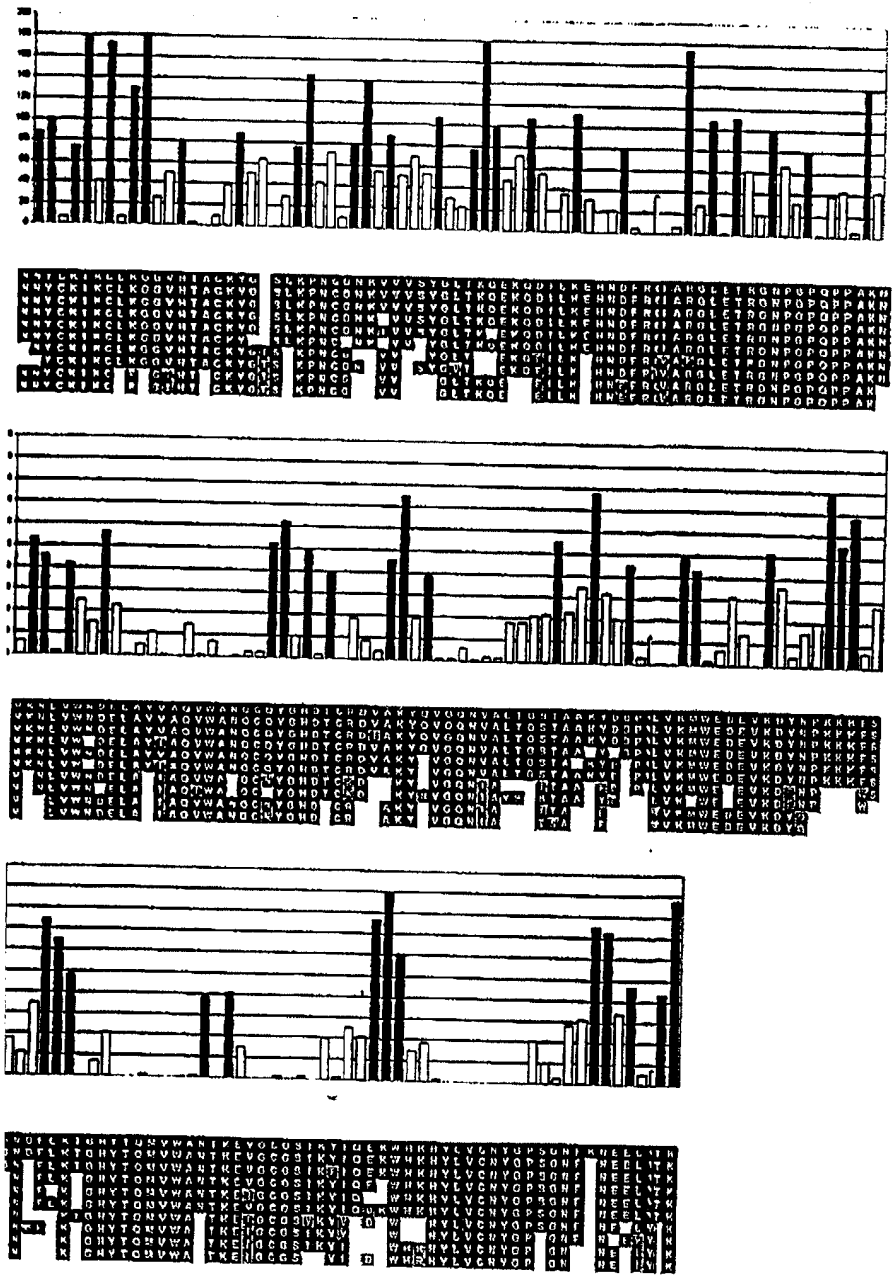

100
80
60
40
20
0
% Inhibition
0,01
0,1
1
10
100
1000
10000
ng inhibitor
Bet v 1
Glu45Ser, Pro108Gly, Asn28Thr+Lys32Gln.

Fig. 9



Conserved residues among Vespula antigen 5

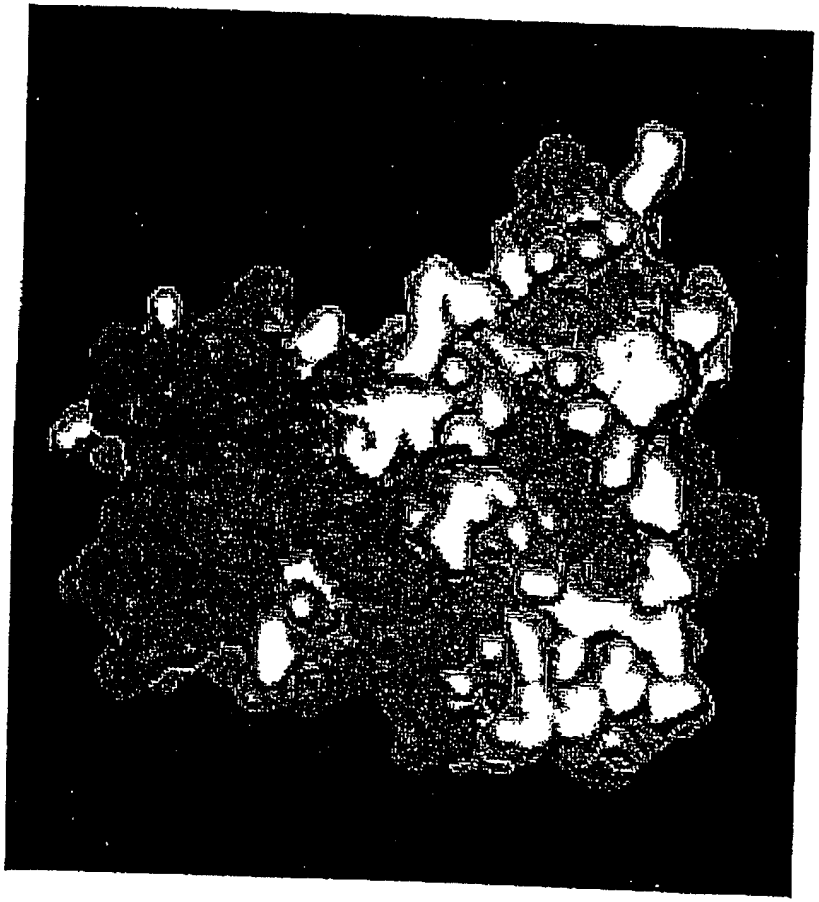


Figure 10.

Fig.11

Mutant-specific oligonucleotide primers used for Ves v 5 mutants. Mutated nucleotides underlined.

Ves v 5 mutant 1 (K72A)

Ves v 5 sense	5´-	ACCACAGCCTCCAGCGAAGAATATGAAAAATTTGGTATGGA -3´
Ves v 5 non-sense	3´-	TGGTGTCGGAGGTCGCTTCTTATACTTTTTAAACCATACCT -5´
sense primer	5´-	CCAGCGGCTAATATGAAAAAT -3´
non-sense primer	3´-	GTCGGAGGTCGCCGATTATAC -5´

Ves v 5 mutant 2 (Y96A)

Ves v 5 sense	5´-	GGCTAATCAATGTCAATATGGTCACGATACTTGCAGGGATG -3´
Ves v 5 non-sense	3´-	CCGATTAGTTACAGTTATACCAGTGCTATGAACGTCCCTAC -5´
sense primer	5´-	TGTCAAGCTGGTCACGATACT -3´
non-sense primer	3´-	TTAGTTACAGTTCGACCAGTG -5´

Fig. 12

Oligonucleotide primers for site directed mutagenesis of Ves v 5.

all sense 1: XhoI start, 38-mer:

EcoRI
5´-CCGCTCGAGAAAAGAAACAATTATTGTAAAATAAAATG
L E K R N N Y C K I K .
Kex2 cleavage site amino terminus of Ves v 5

1	sense	1: K72As	21-mer	5´-CCAGCGGCTAATATGAAAAAT
1	non-sense	2: K72Aa	21-mer	5´-CATATTAGCCGCTGGAGGCTG
2	sense	3: Y96As	21-mer	5´-TGTCAAGCTGGTCACGATACT
2	non-sense	4: Y96Aa	21-mer	5´-GTGACCAGCTTGACATTGATT
all	non-sense	7: CT-pPICZαA,	21-mer	5`-ATTCATCAGCTGCGAGATAGG

Fig. 13

Overview of Ves v 5 mutations

```
   1 AACAATTATTGTAAAATAAAATGTTTGAAAGGAGGTGTCCATACTGCCTGCAAATATGGA   60
   1  N  N  Y  C  K  I  K  C  L  K  G  G  V  H  T  A  C  K  Y  G    20

  61 AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCTAACGAAACAAGAG  120
  21  S  L  K  P  N  C  G  N  K  V  V  V  S  Y  G  L  T  K  Q  E    40

 121 AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAATTGCACGAGGATTGGAG  180
  41  K  Q  D  I  L  K  E  H  N  D  F  R  Q  K  I  A  R  G  L  E    60

                                                1[K72A] (AAG-GCT)
 181 ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAATATGAAAAATTTGGTATGGAAC  240
  61  T  R  G  N  P  G  P  Q  P  P  A  K  N  M  K  N  L  V  W  N    80

                                                2[Y96A] (TA-GC)
 241 GACGAGTTAGCTTATGTCGCCCAAGTGTGGGCTAATCAATGTCAATATGGTCACGATACT  300
  81  D  E  L  A  Y  V  A  Q  V  W  A  N  Q  C  Q  Y  G  H  D  T   100

 301 TGCAGGGATGTAGCAAAATATCAGGTTGGACAAAACGTAGCCTTAACAGGTAGCACGGCT  360
 101  C  R  D  V  A  K  Y  Q  V  G  Q  N  V  A  L  T  G  S  T  A   120

 361 GCTAAATACGATGATCCAGTTAAACTAGTTAAAATGTGGGAAGATGAAGTGAAAGATTAT  420
 121  A  K  Y  D  D  P  V  K  L  V  K  M  W  E  D  E  V  K  D  Y   140

 421 AATCCTAAGAAAAAGTTTTCGGGAAACGACTTTCTGAAAACCGGCCATTACACTCAAATG  480
 141  N  P  K  K  K  F  S  G  N  D  F  L  K  T  G  H  Y  T  Q  M   160

 481 GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATAAAATACATTCAAGAGAAATGG  540
 161  V  W  A  N  T  K  E  V  G  C  G  S  I  K  Y  I  Q  E  K  W   180

 541 CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTAAGAATGAGGAACTT  600
 181  H  K  H  Y  L  V  C  N  Y  G  P  S  G  N  F  K  N  E  E  L   200

 601 TATCAAACAAAGTAA                                               612
 201  Y  Q  T  K  stop                                              204
```

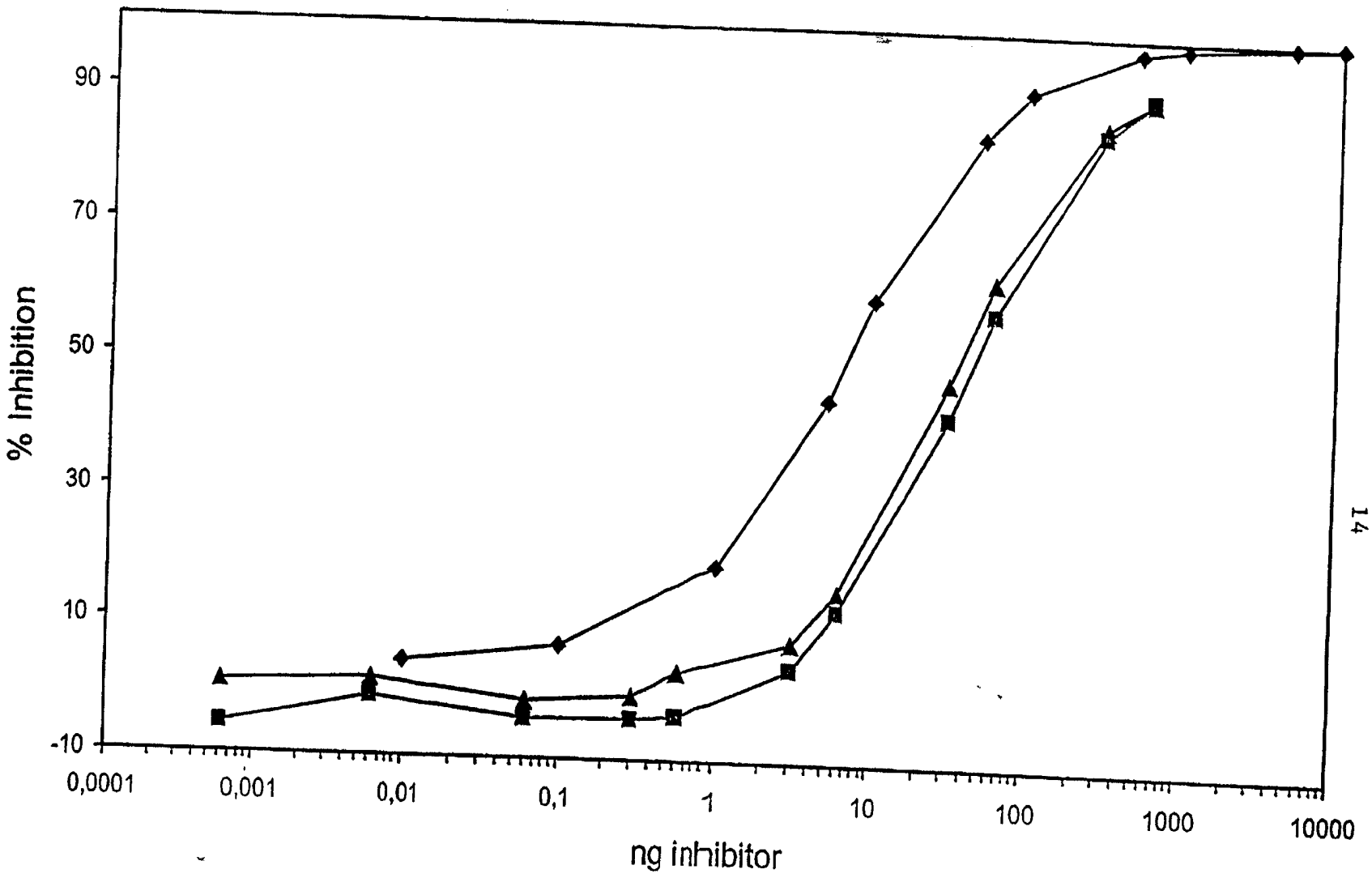
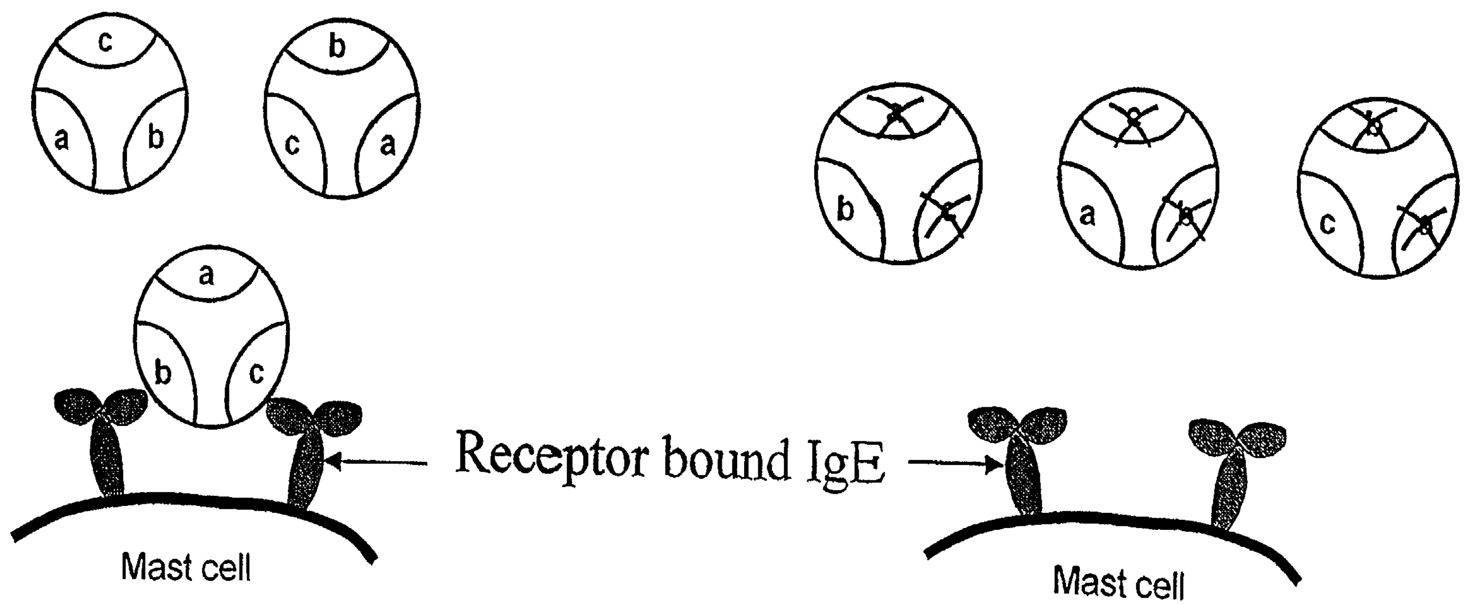

90
70
50
30
10
-10
% Inhibition
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
ng inhibitor
r Ves v 5
Lys72Ala
Tyr96Ala

Fig. 14

Fig. 15

Effect of point mutations in dominating IgE epitopes
hypothetical model with 3 epitopes



Cross-linking Fig. 15A

No cross-linking Fig. 15B

Fig. 16

DNA SEQUENCE

Der p 2 (DNA sequence referred to in notes in accession No. P49278 SWISSPROT)

ORIGIN

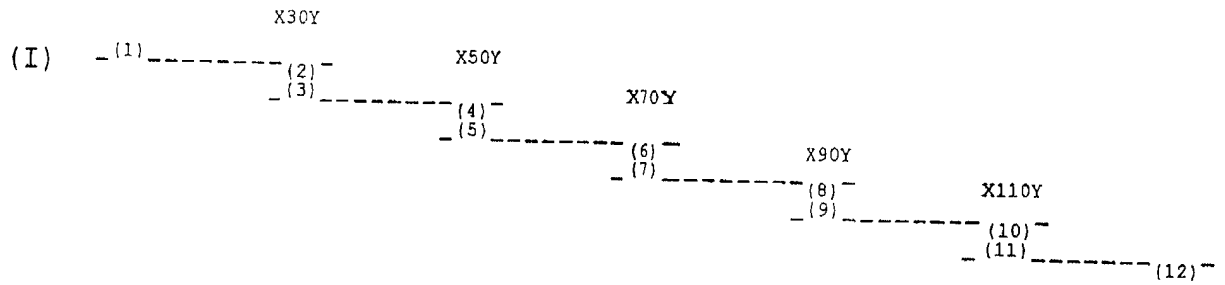
1 cacaaattct tctttcttcc ttactactga tcattaatct gaaaacaaaa ccaaacaaac
61 cattcaaaat gatgtacaaa attttgtgtc tttcattgtt ggtcgcagcc gttgctcgtg
121 atcaagtcga tgtcaaagat tgtgccaatc atgaaatcaa aaaagttttg gtaccaggat
181 gccatggttc agaaccatgt atcattcatc gtggtaaacc attccaattg gaagccgttt
241 tcgaagccaa ccaaaacaca aaaacggcta aaattgaaat caaagcctca atcgatggtt
301 tagaagttga tgttcccggt atcgatccaa atgcatgcca ttacatgaaa tgcccattgg
361 ttaaaggaca acaatatgat attaaatata catggaatgt tccgaaaatt gcaccaaaat
421 ctgaaaatgt tgtcgtcact gttaaagtta tgggtgatga tggtgttttg gcctgtgcta
481 ttgctactca tgctaaaatc cgcgattaaa tcaaacaaaa tttattgatt ttgtaatcac
541 aaatgattga ttttctttcc aaaaaaaaaa taaataaaat tttgggaatt c

AMINO ACID SEQUENCE

Der p 2 (Accession No. P49278 SWISSPROT; includes signal peptide 1-17)

1 mmykilclsl lvaavardqv dvkdcanhei kkvlvpgchg sepciihrgk pfqleavfea
61 nqntktakie ikasidglev dvpgidpnac hymkcplvkg qqydikytwn vpkiapksen
121 vvvtvkvmgd dgvlacaiat hakird

Figure 17

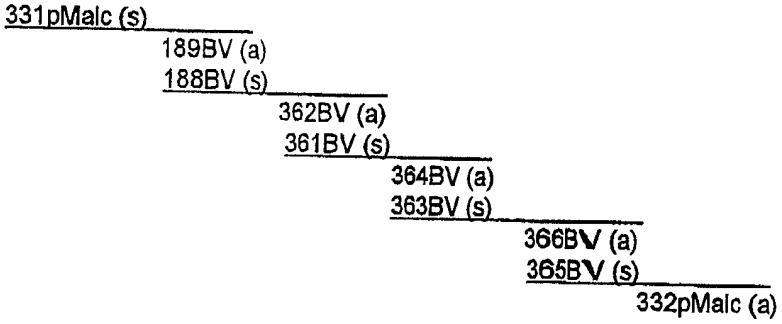


Lines represents DNA sequences.
Numbers in parentheses above lines represents sense oligonucleotide primers: (1), (3), (5), (7), (9), (11).
Numbers in parentheses below lines represents anti-sense oligonucleotide primers: (2), (4), (6), (8), (10), (12).
Notation X (position) Y represents mutations.
(1) Represents the sense oligonucleotide primer accommodating the protein N-terminus.
(12) Represents the anti-sense oligonucleotide primer accommodating the protein C-terminus.

Figure 18

Bet v 1 (2628) (Y5V, E45S, K65N, K97S, K134E)

DNA template: Bet v 1 (2589) carrying the Y5V mutation.

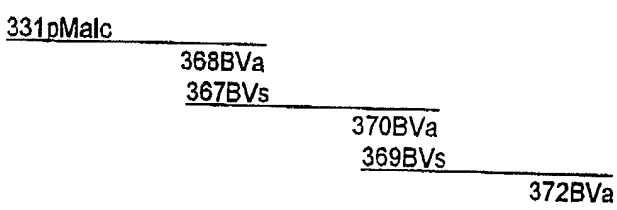



```
331pMal c : CAGACTAATTCGAGCTCGGTACCC
189BV     : TTTCCTGAAATGTTTTCAACACT
188BV     : AACATTTCAGGAAATGGAGGGCC
362Bva    : CACGTAGTTGAAAGGGAGGCCTTC
361BVs    : TTTCAACTACGTGAAGGACAGAGT
364Bva    : GGAGATGCTCTCCAATGTGTCGCC
363BVs    : GGAGAGCATCTCCAACGAGATAAA
366Bva    : ACTTGCTTCAACCTGCTCTGCCTT
365BVs    : CAGGTTGAAGCAAGTAAAGAAATG
332pMal c : GCAGGTCGACTCTAGAGGATCCAT
```

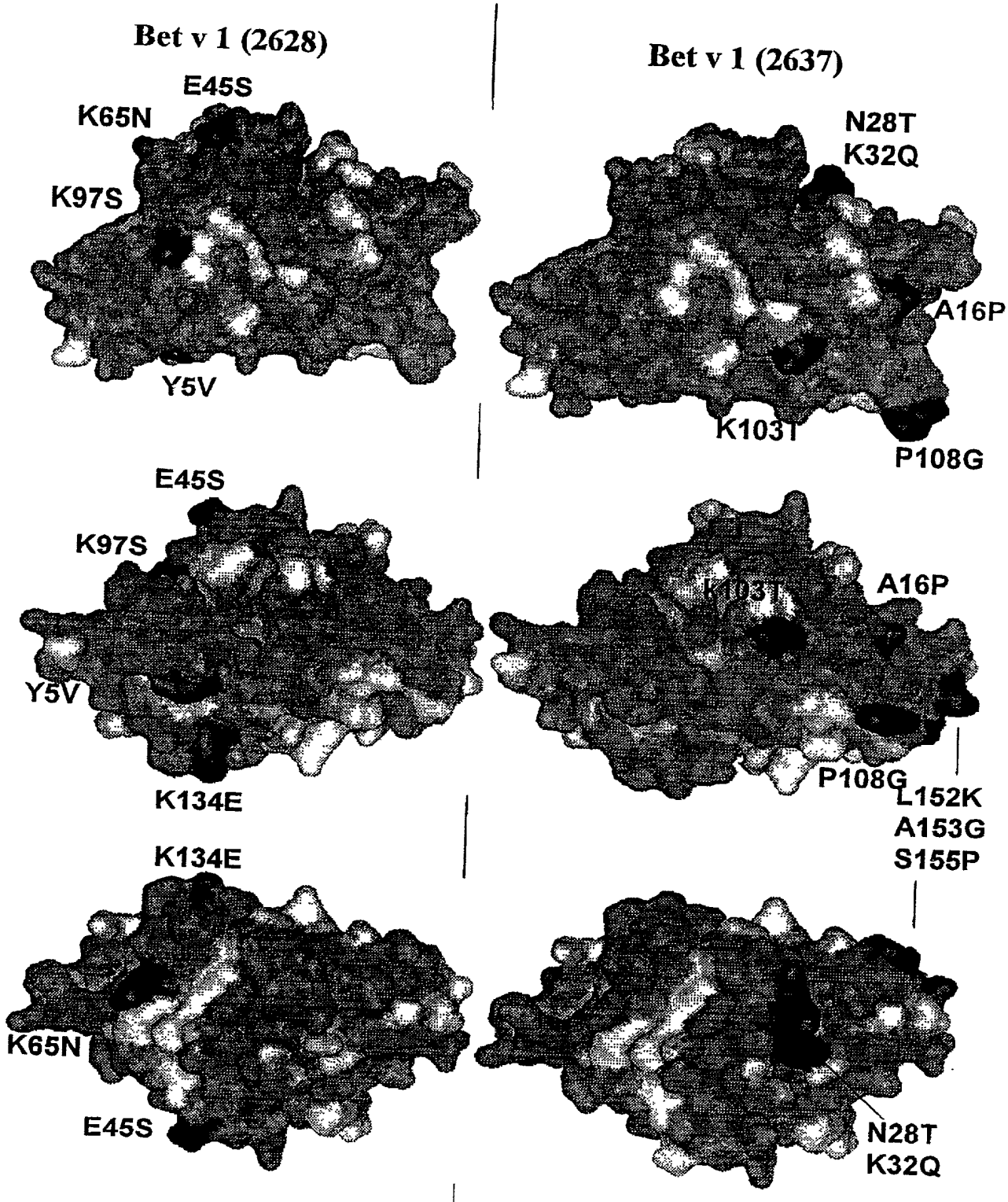
Bet v 1 (2637)

(A16P, N28T, K32Q, K103T, P108G, L152K, A153G, S155P)

DNA template: Bet v 1 (2571) carrying N28T, K32Q, P108G mutations.



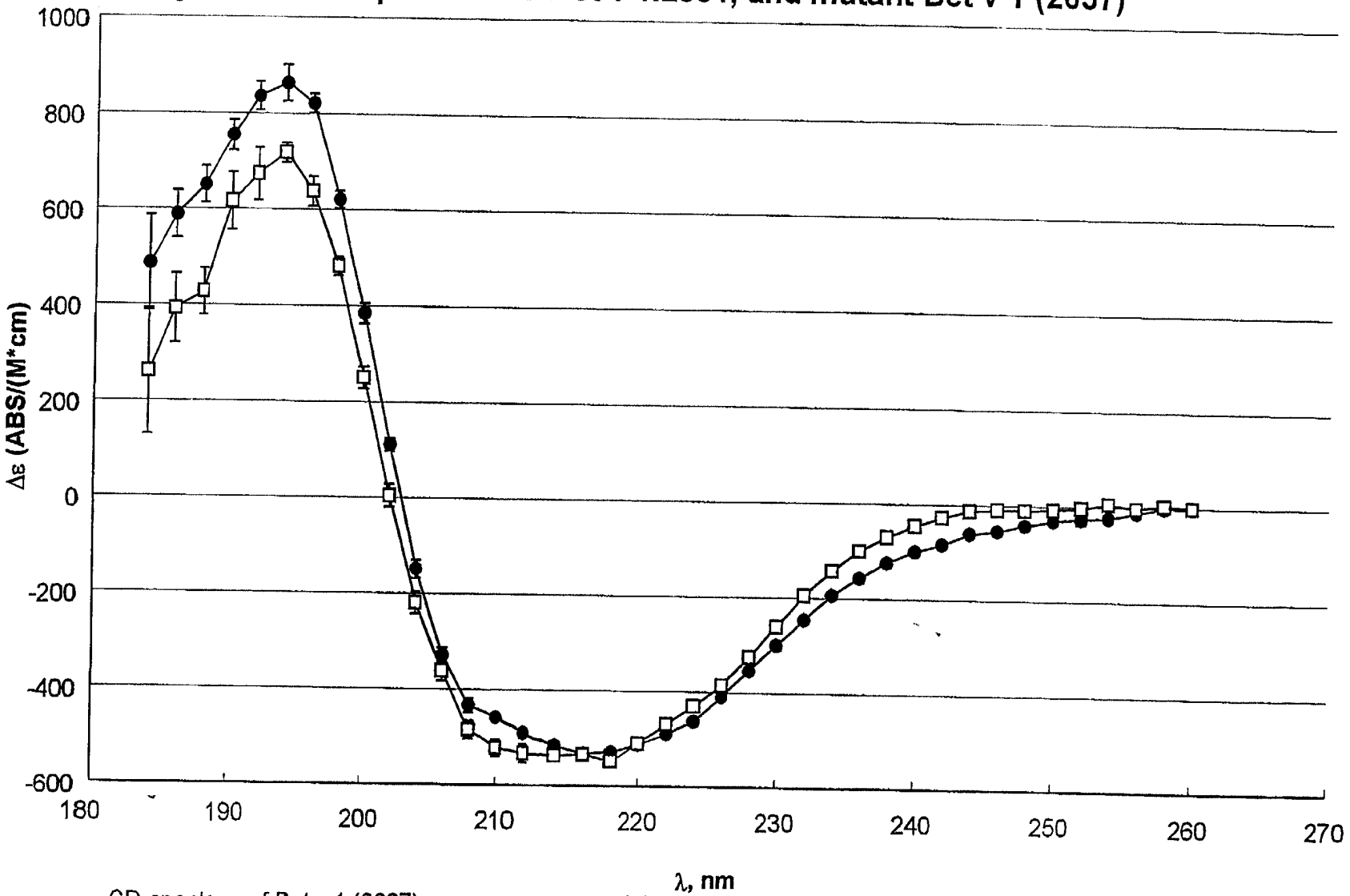

```
331pMalc    :CAGACTAATTCGACGTCGGTACCC
368BVa      :CAGTCGcggTGCTGGGATAACAGA
367BVs      :CCAGCAccgCGACTGTTCAAGGCC
370BVa      :CACTATggtTATCTCGTTGGAGAT
369BVs      :GAGATAaccATAGTGGCAACtggT
372BVa:TTACTGAATTCATTAGTTGTAGGCATCcggGTGgcctttGAGGTA
```



Molecular surface of Bet v 1.
Left side: Bet v 1 (2628), Right side: Bet v 1 (2637)
Grey: Backbone + amino acids 95-100% conserved among *Fagales*
Black: Introduced point mutations.

Figure 19

Figure 20 **CD-spectrum of Bet v 1.2801, and mutant Bet v 1 (2637)**



CD-spectrum of Bet v 1 (2637), open squares, and the CD-spectrum of native folded Bet v 1.2801, closed circles, both obtained at 20 °C

Figure 21

Inhibition of human serum IgE-binding to Bet v 1.2801 with Bet v 1.2801 and mutated Bet v 1 allergens

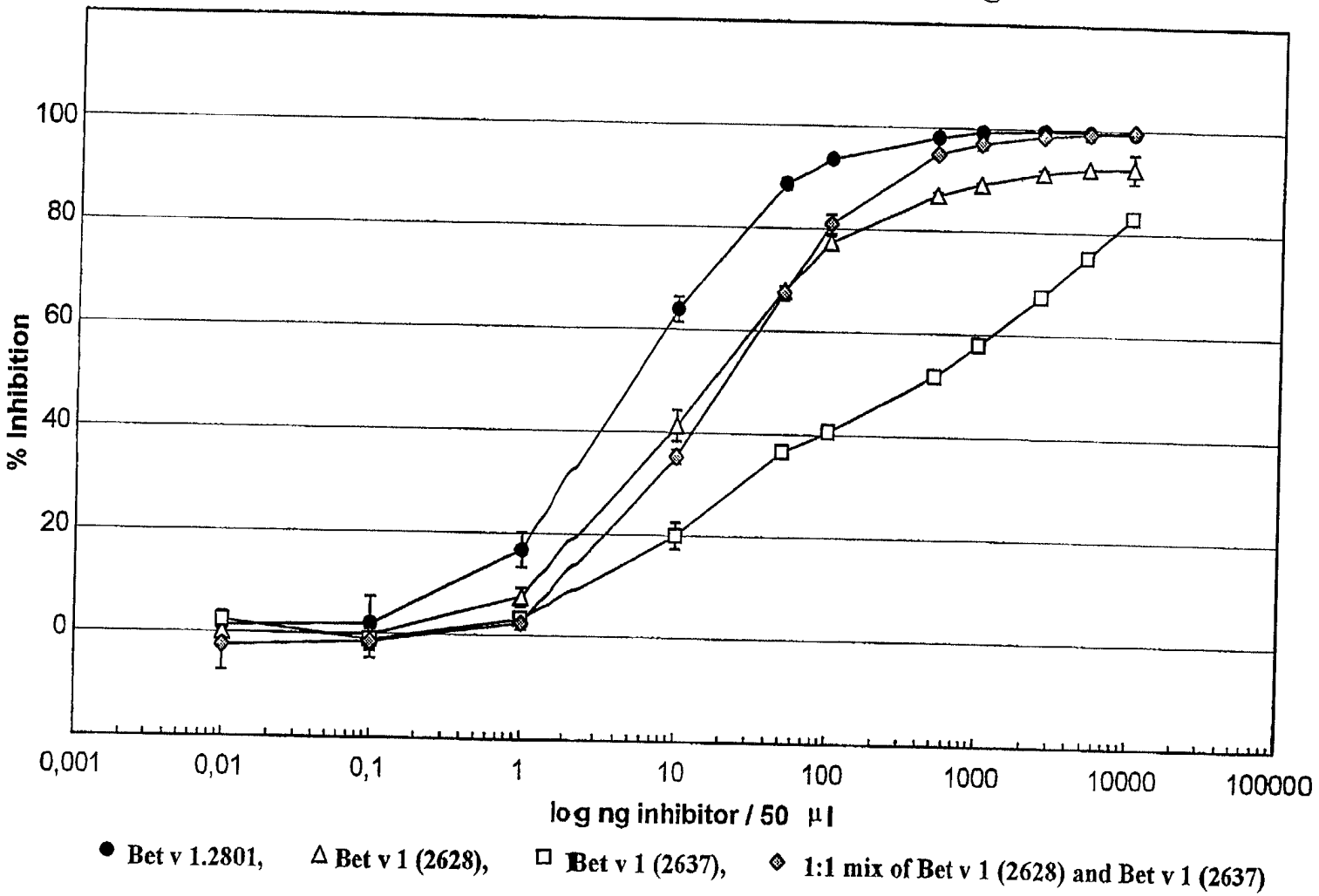


Figure 22 **Histamine release, donor MCDS, Bet v 1.2801, Bet v 1(2628), Bet v 1 (2637)**

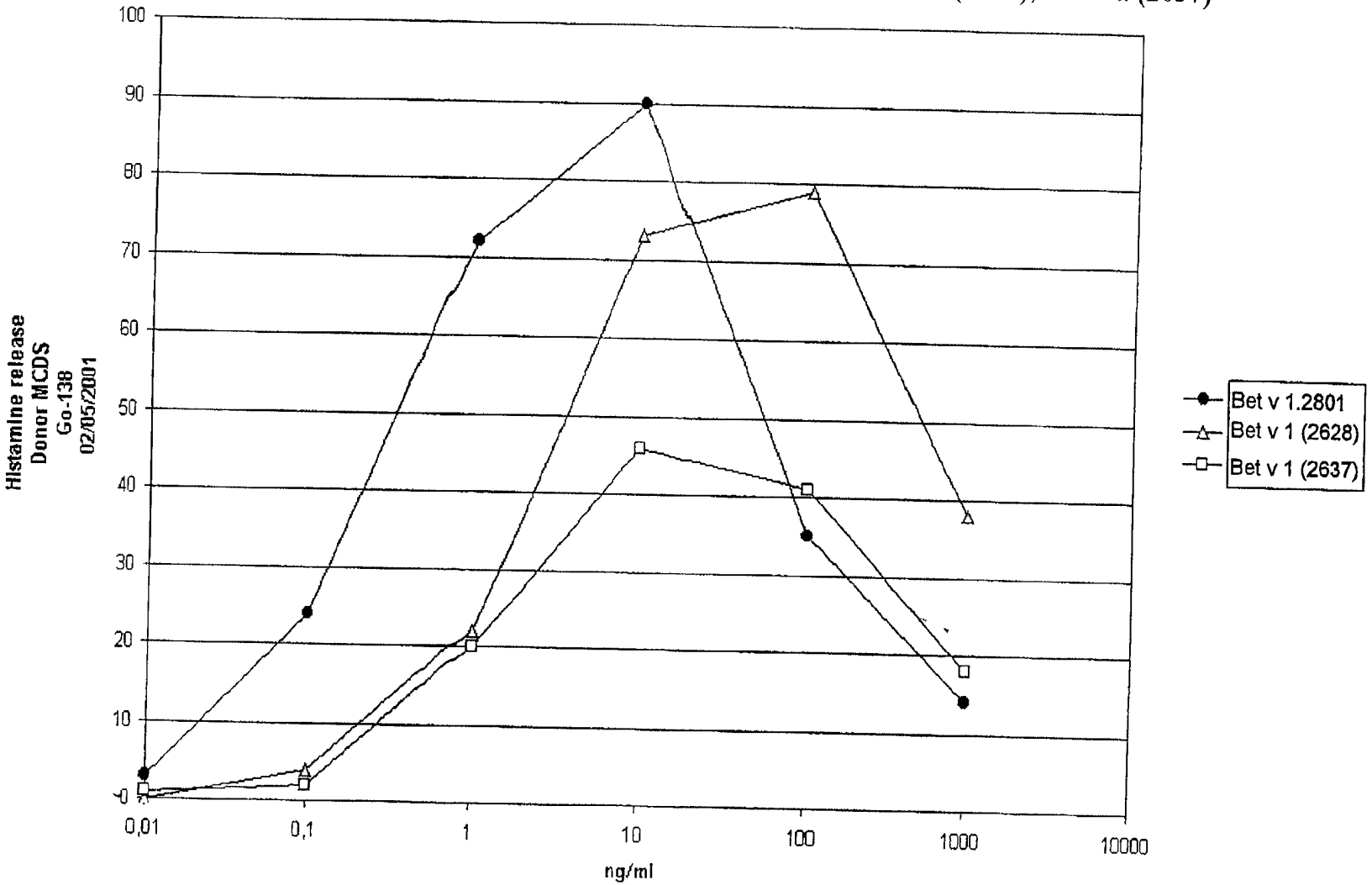


Figure 23

Histamine release, donor MDH, Bet v 1.2801, Bet v 1(2628), Bet v 1 (2637)

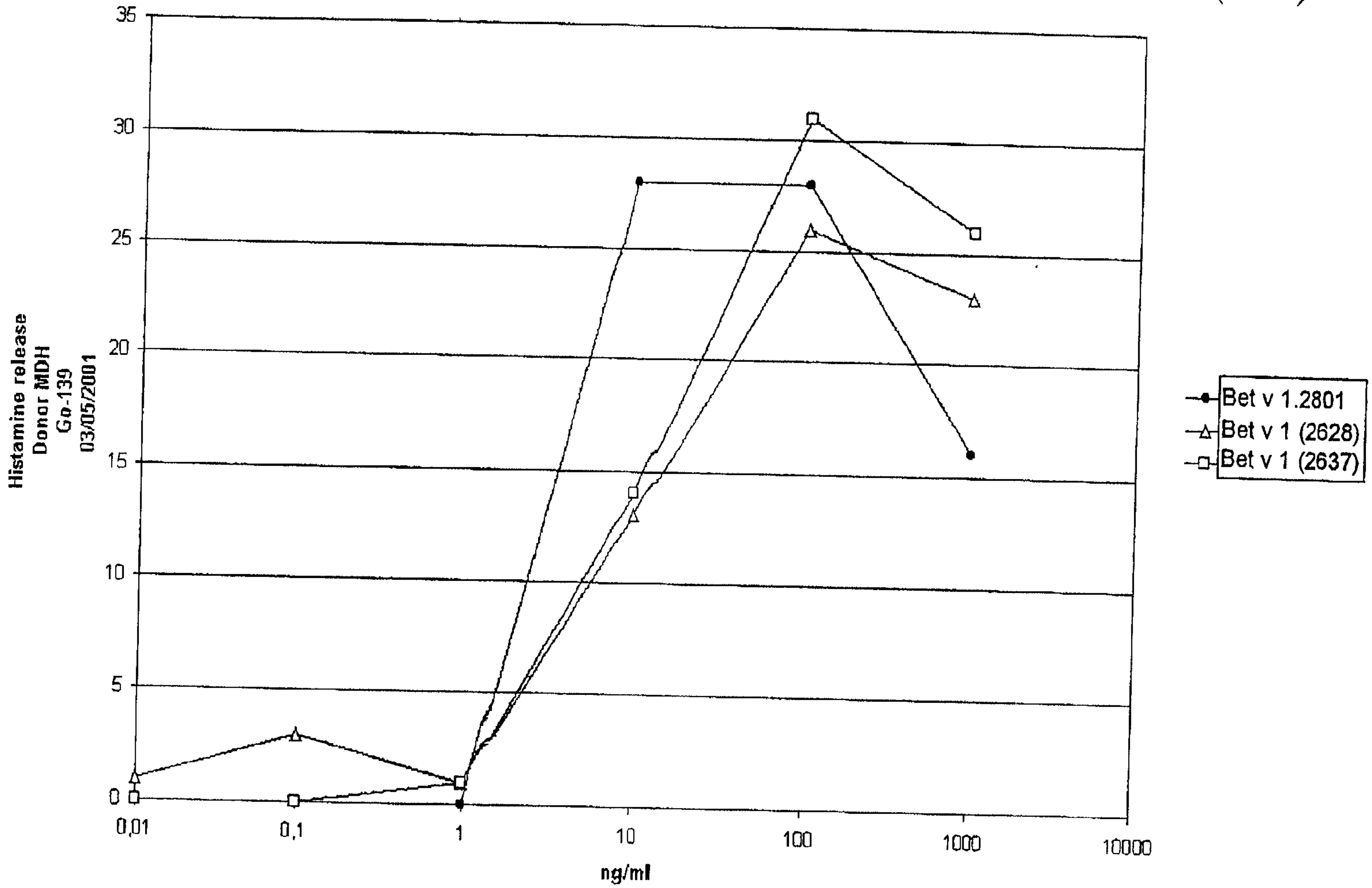
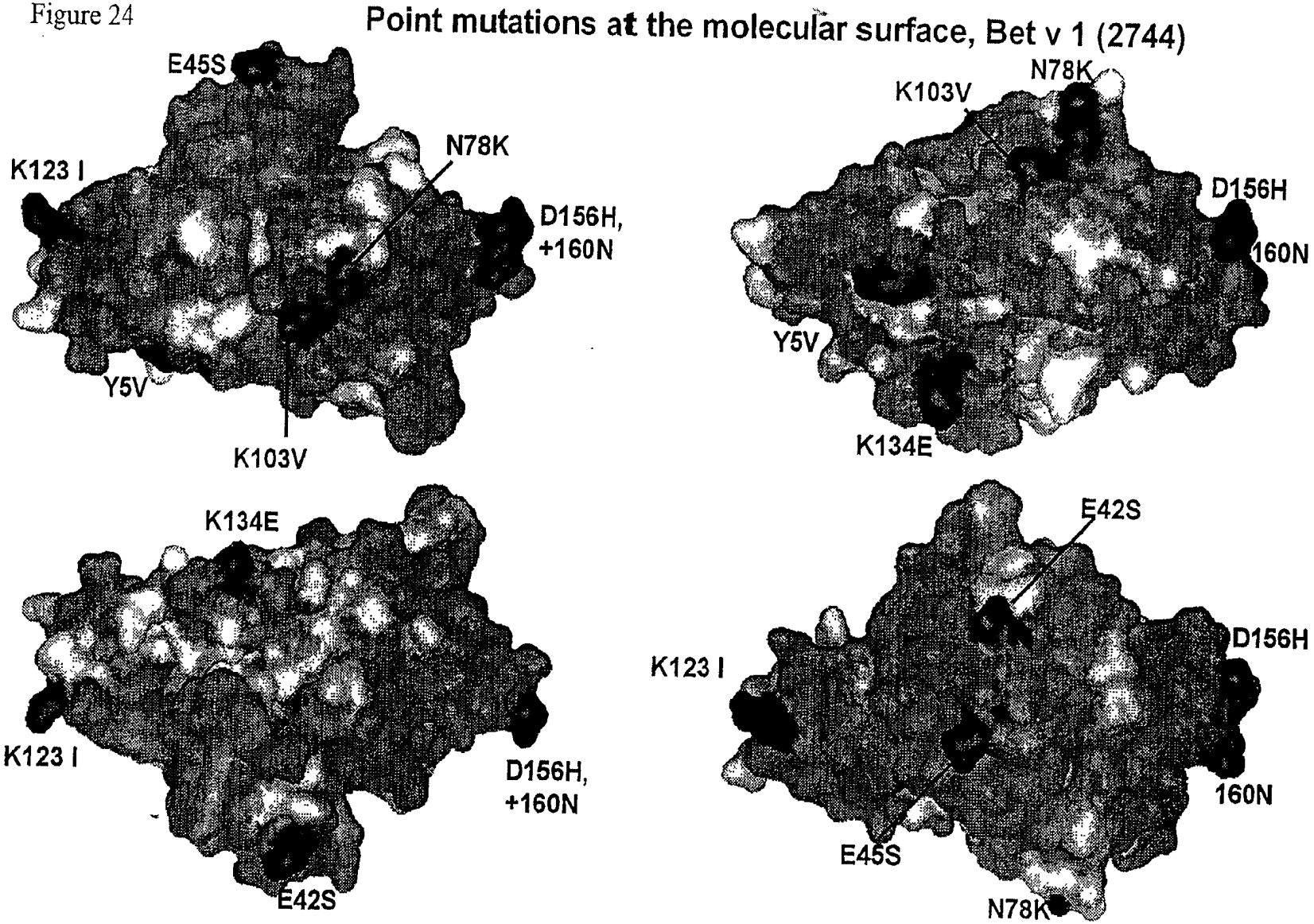
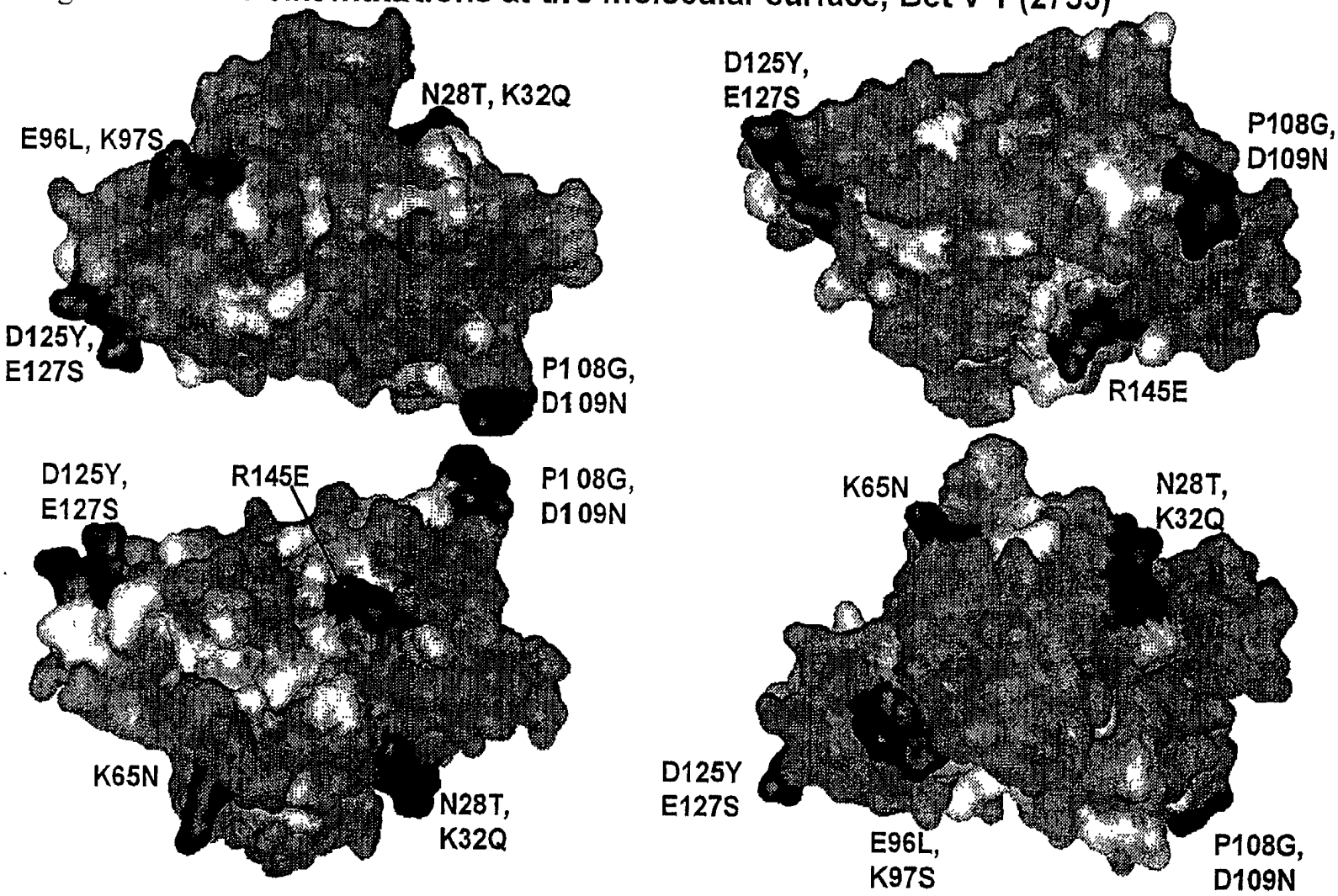


Figure 24



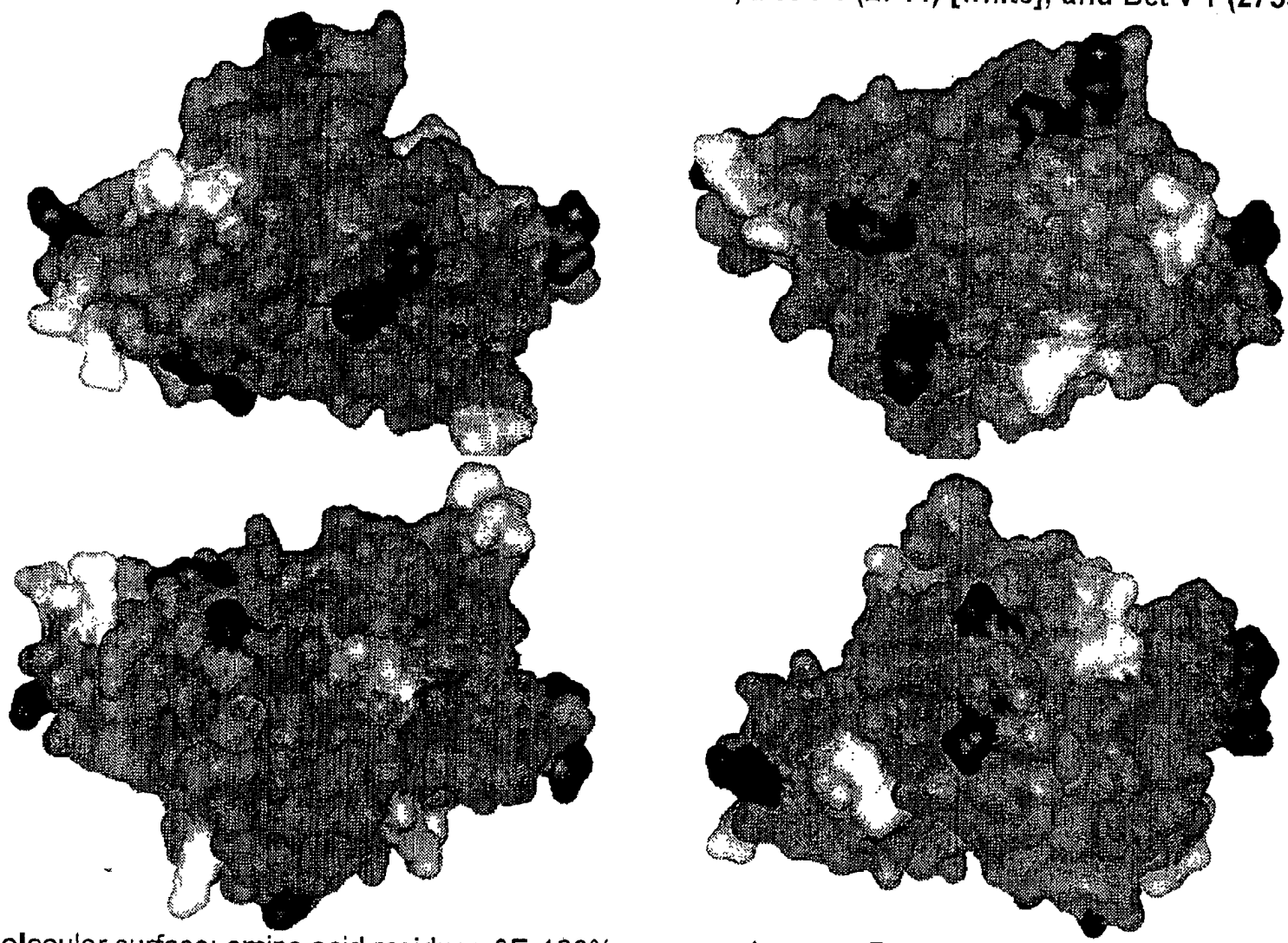
Grey: Back bone + Amino acid residues 95-100 % conserved among *Fagales*, Black: Point mutations

Figure 25 **Point mutations at the molecular surface, Bet v 1 (2753)**



Grey: Back bone + Amino acid residues 95-100% conserved among *Fagales*, Black: Point mutations

Distribution of point mutations at the molecular surface of, Bet v 1 (2744) [white], and Bet v 1 (2753) [Black]



Grey: Molecular surface; amino acid residues 95-100% conserved among *Fagales*
Black: Mutations (Y5V, K134E), (E42S, E45S), (N78K, K103V), K123 I, (D156H, +160N)
White: Mutations (N28T, K32Q), K65N, (E96L, K97S), (P108G, D109N), (D125Y, E127S), R145E

Figure 26

Figure 27 **Circular dichroisme spectra of Bet v 1.2801 and mutant Bet v 1(2744), pH 7.13, T 20C.**

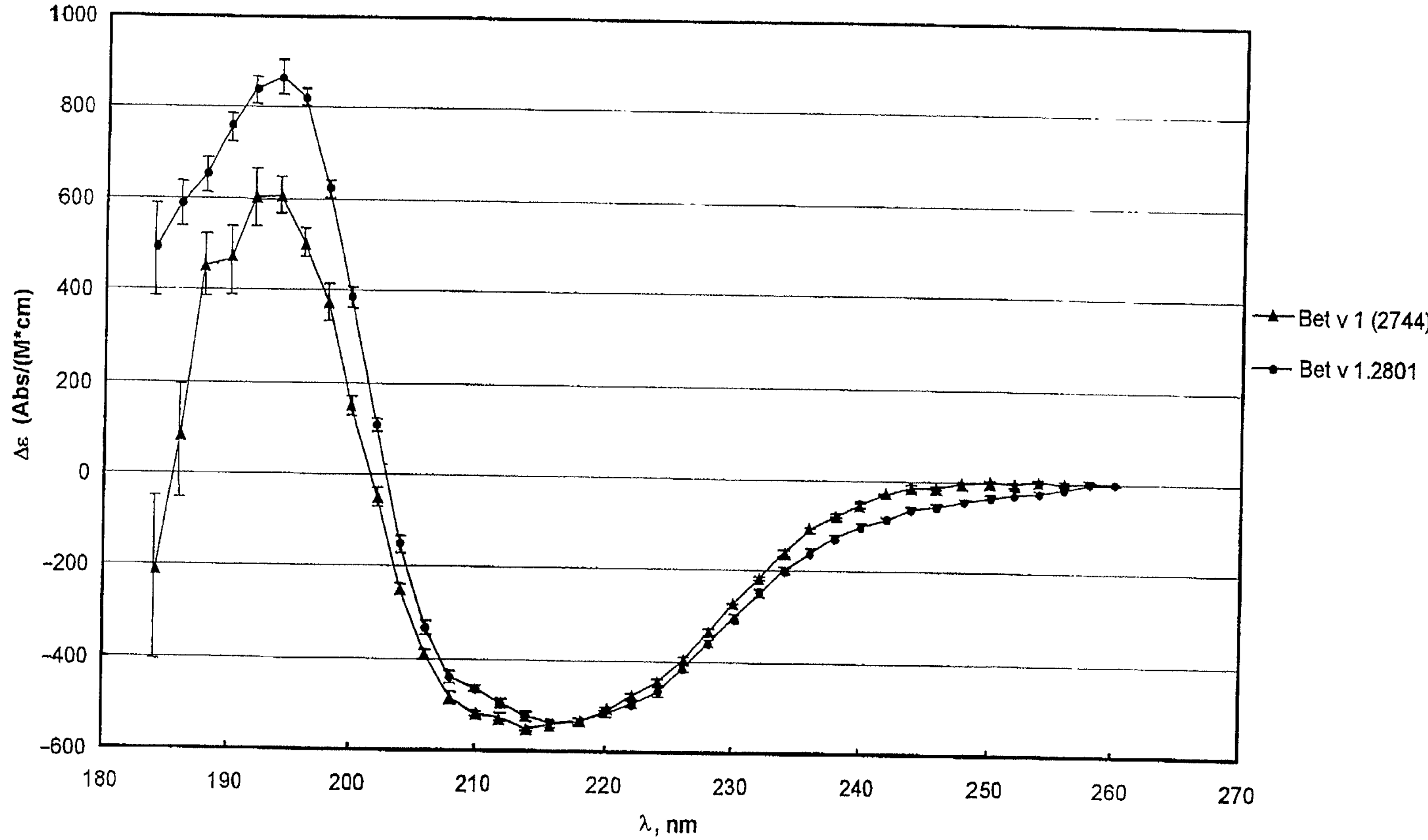


Fig. 28: Histamine release, donor MK, Bet v 1.2801 and Bet v 1(2744)

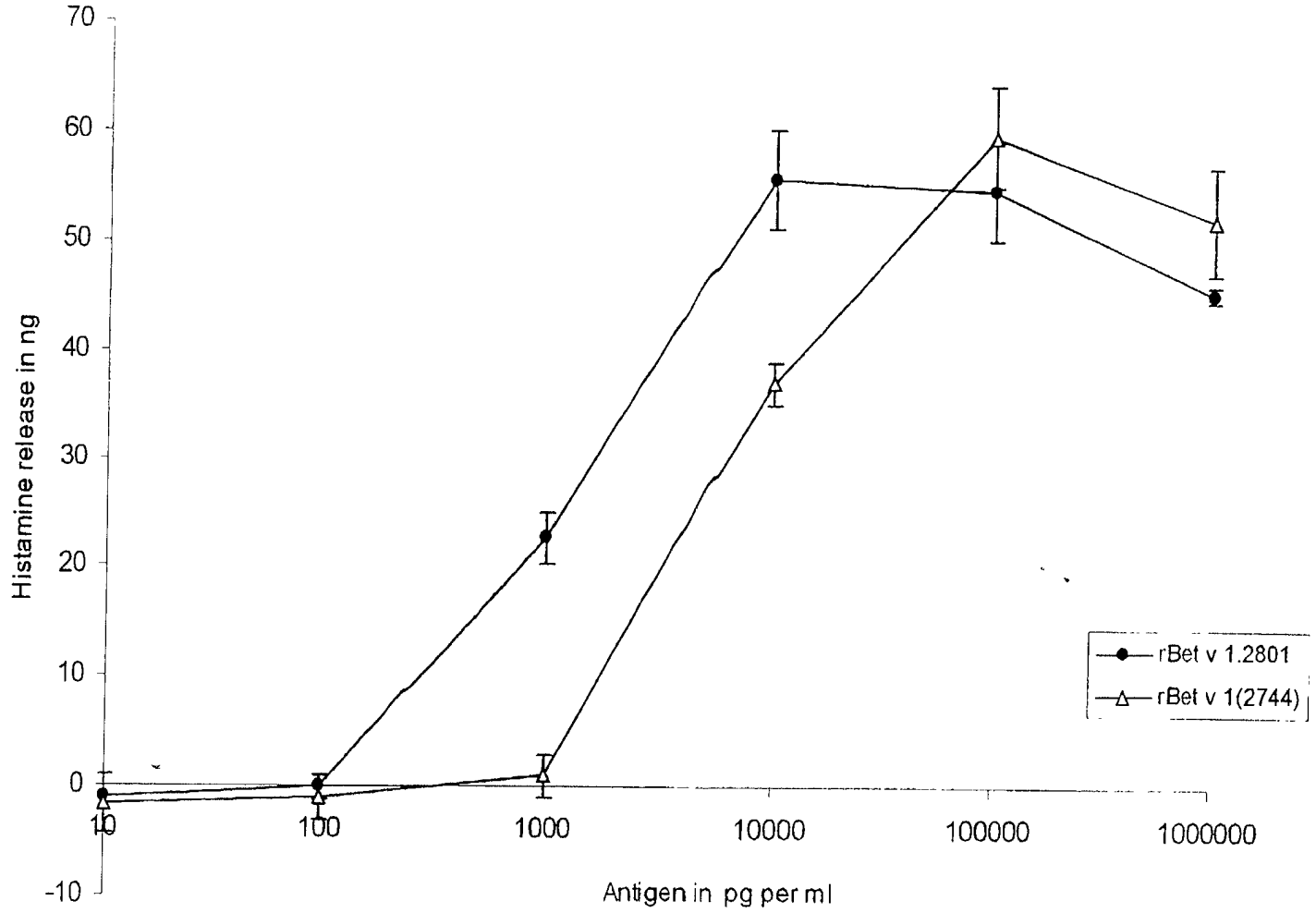


Fig. 29A: Histamine release, donor MJ, Bet v 1.2801 and Bet v 1 (2744)

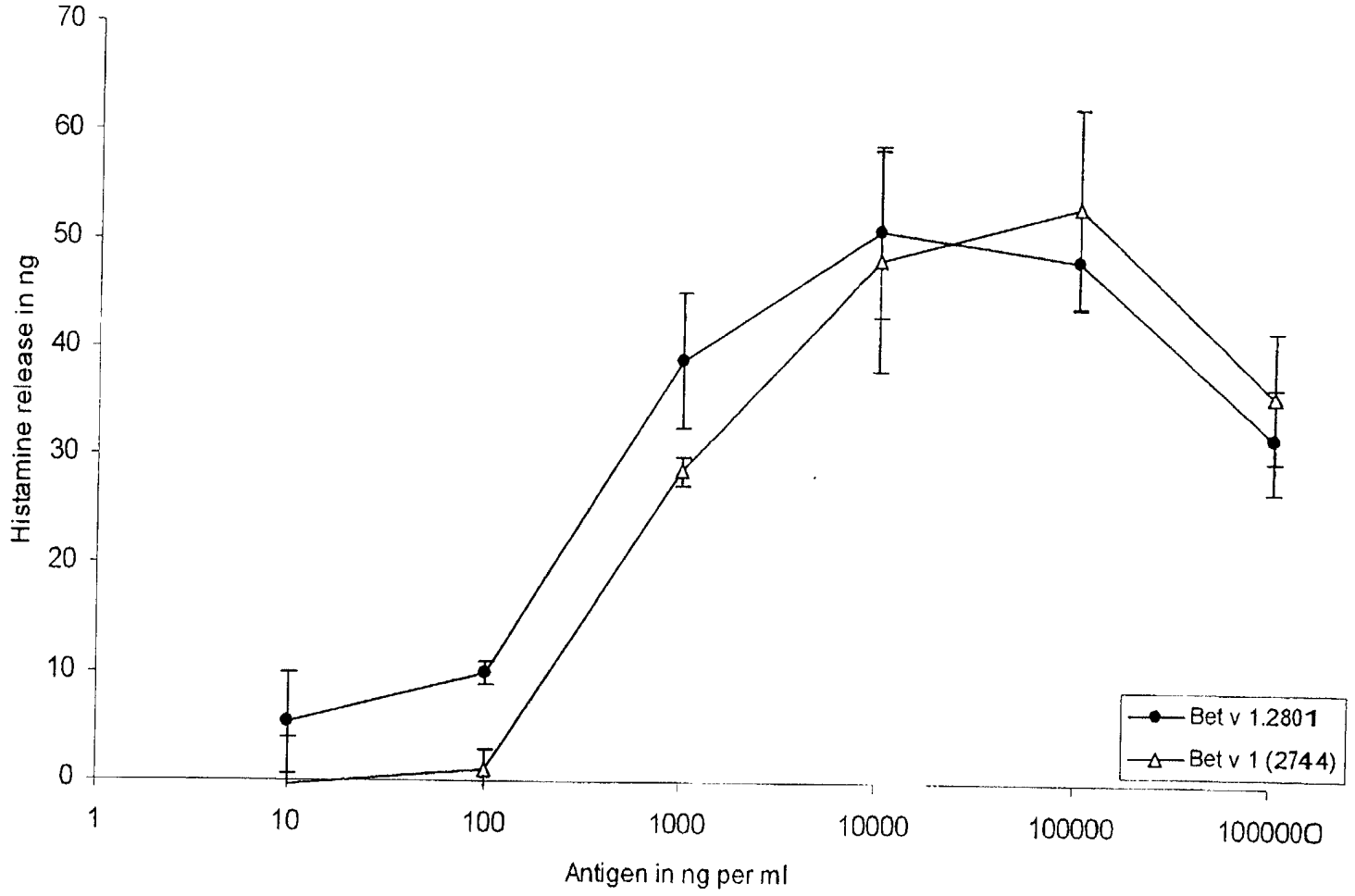


Fig.29B: Histamine release, donor MH, Bet v 1.2801 and Bet v 1 (2744)

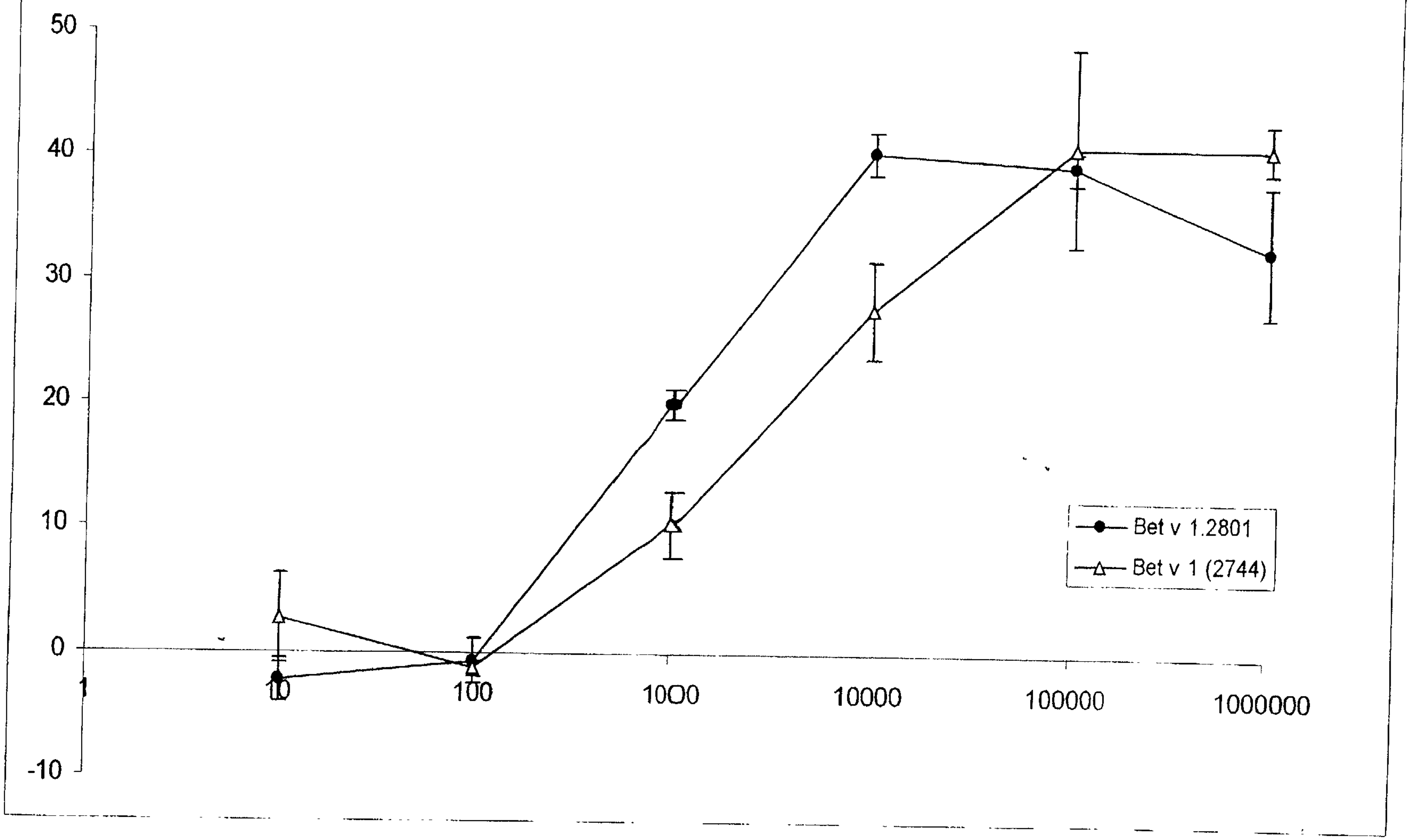


Fig. 29C: Histamine release, donor CJB, Bet v 1.2801 and Bet v 1 (2744)

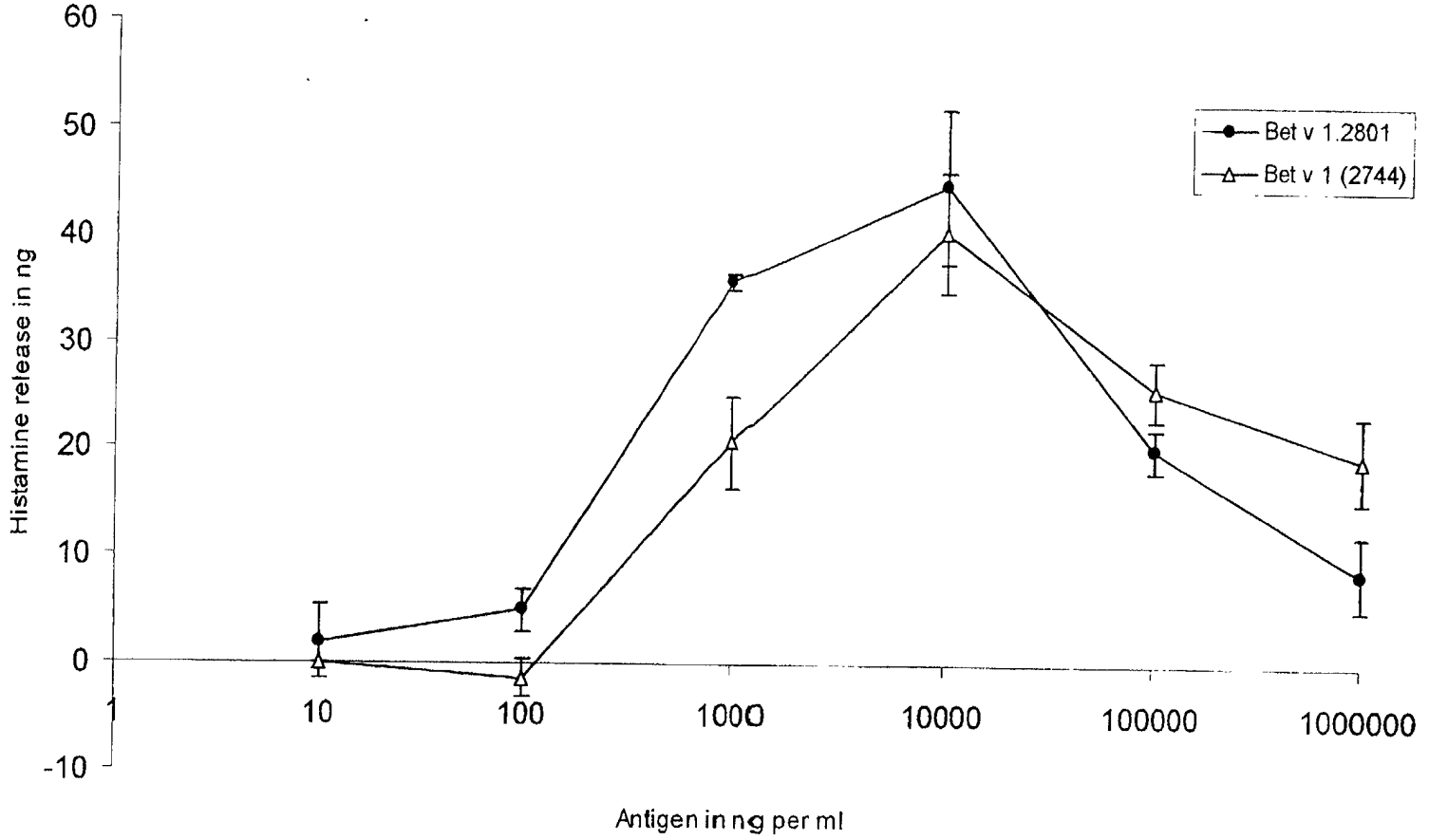


Fig. 29D: Histamine release, donor MCDS, Bet v 1.2801 and Bet v 1 (2744)

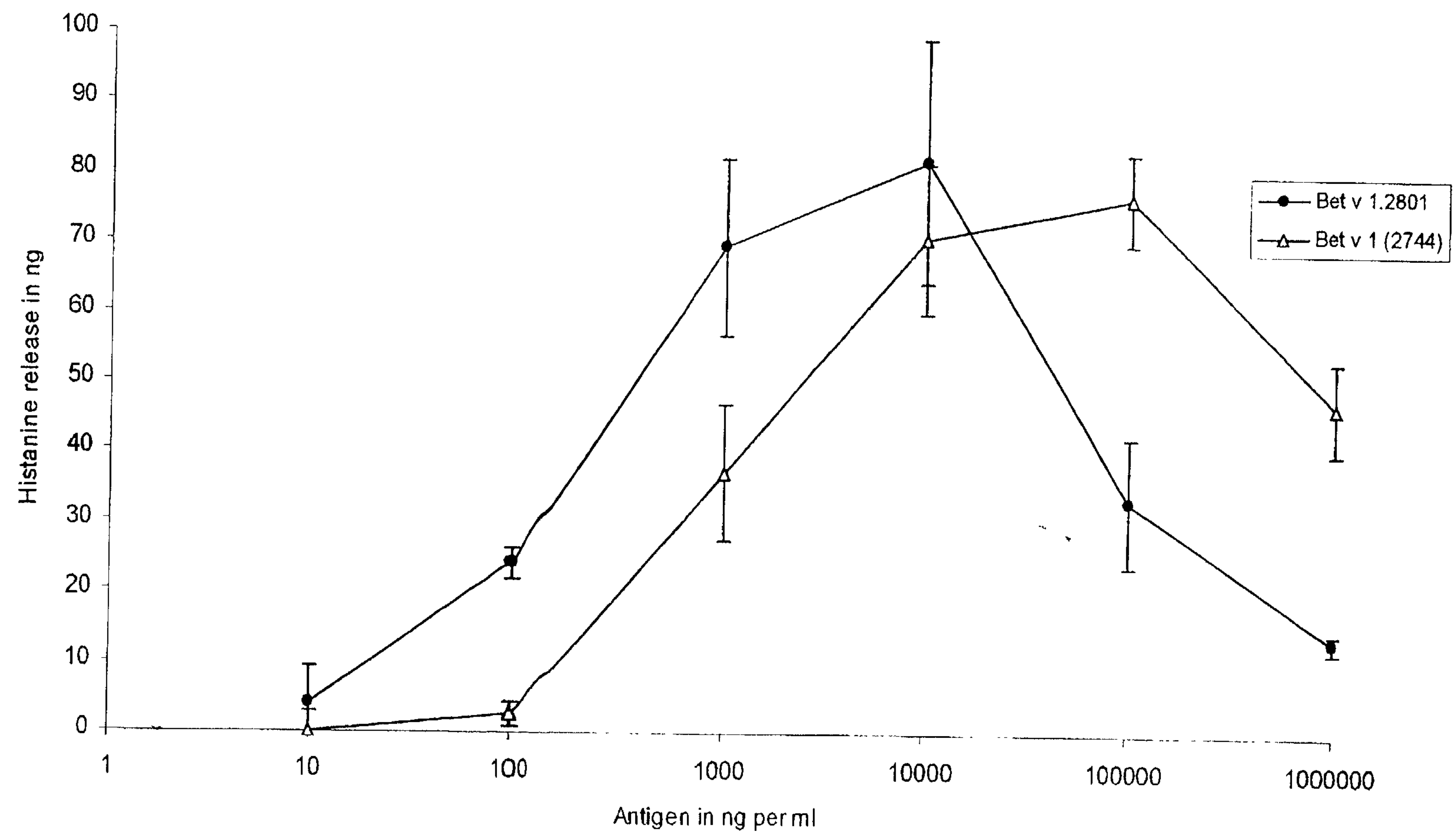
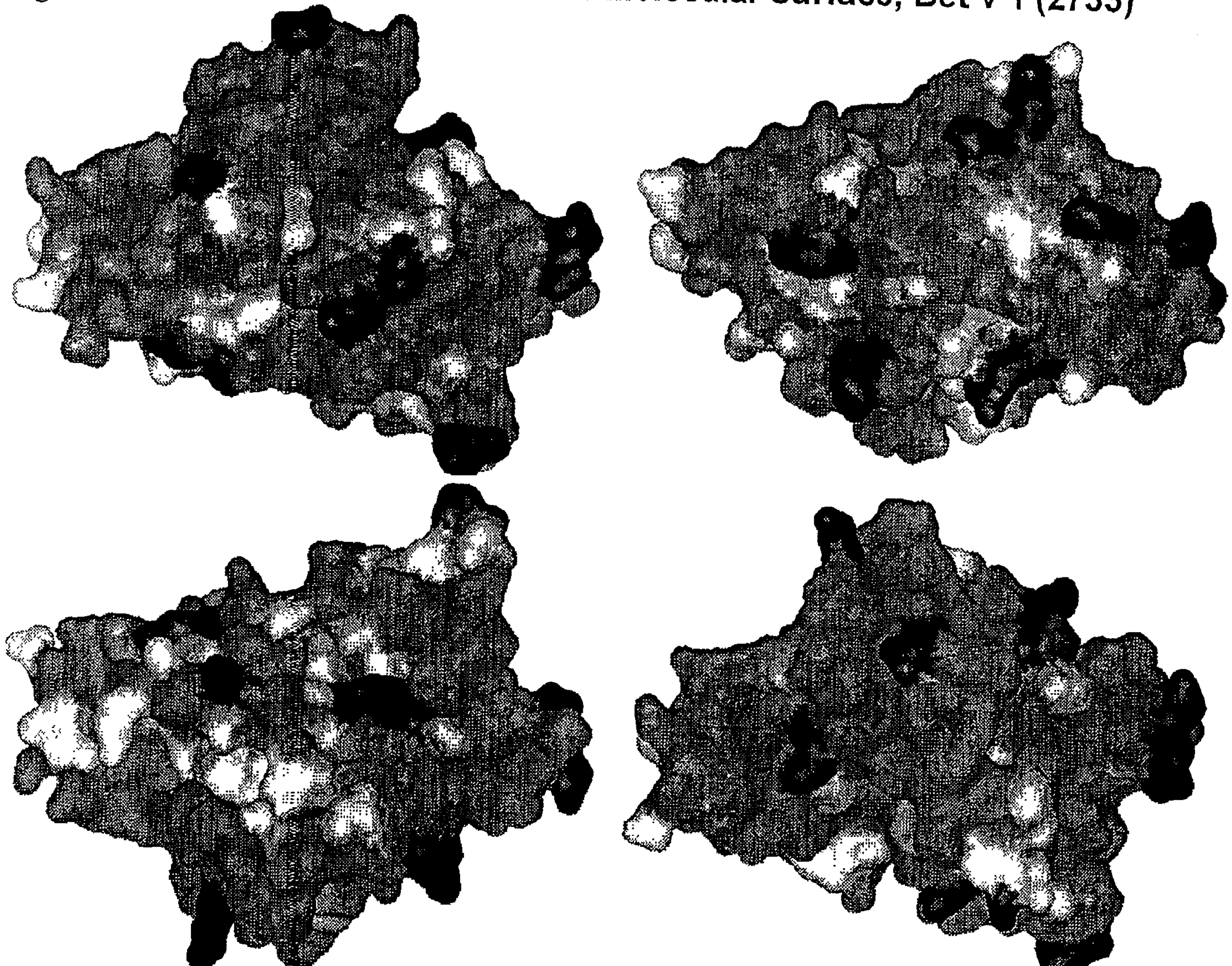


Figure 30 **Point mutations at the Molecular surface, Bet v 1 (2733)**



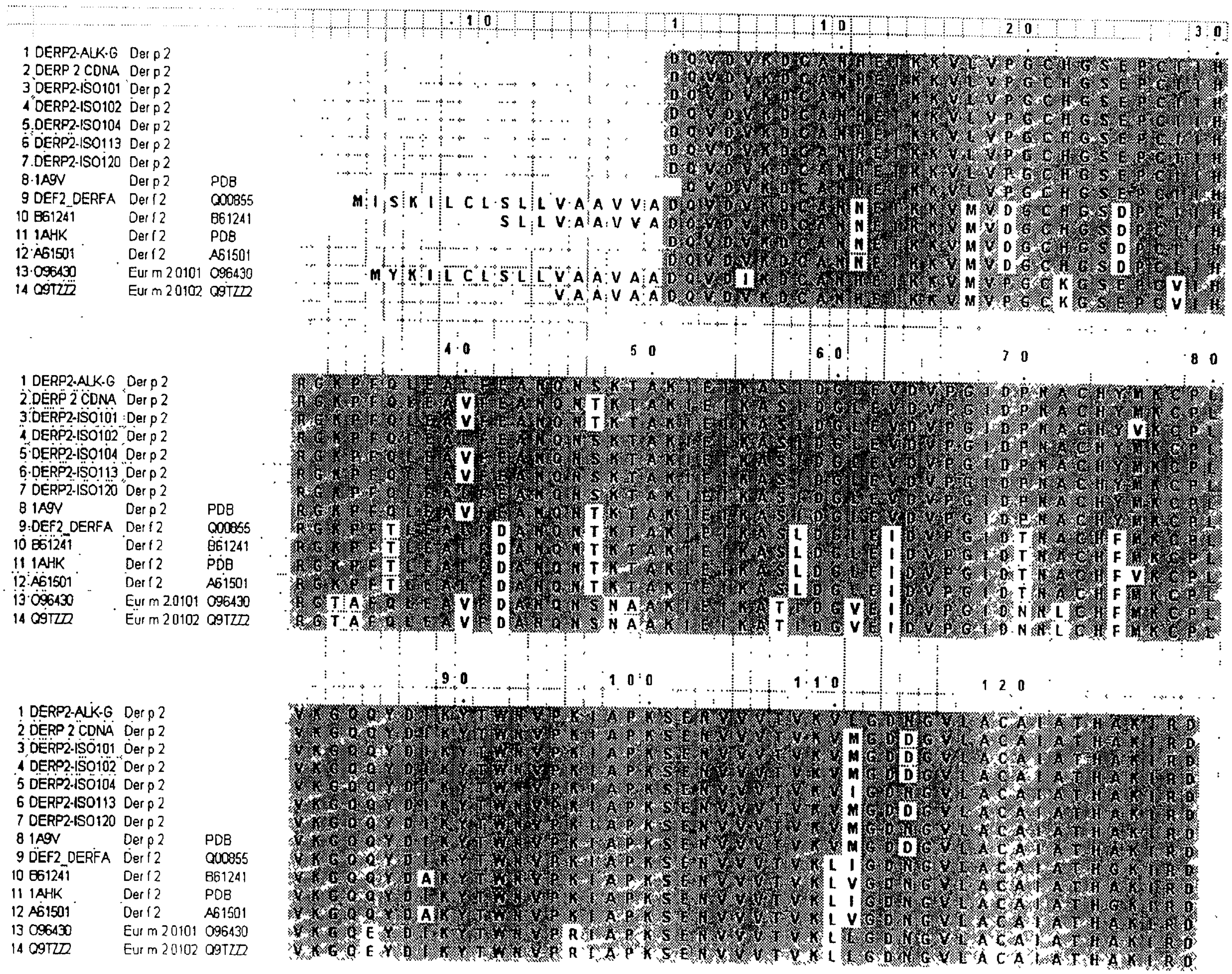
Grey: Back bone + Amino acid residues 95-100% conserved among *Fagales*,
Black: Point mutations: Y5V, N28T, K32Q, E45S, K65N, N78K, K97S, K103V, P108G, K134E, R145E, D156H, +160N

Figure 31

Oligonucleotide primers for site-directed mutagenesis of Der p 2

K6A	sense	OB43	42-mer	5' -CCG*Xho I*CTCGAGAAAAGAGATCAAGTCGATGTCGCCGATTGTGCC- 3'
	anti-sense	OB28	39-mer	5' -CGT*Xba I*CTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
K15E	sense	OB44	67-mer	5' -CCG*Xho I*CTCGAGAAAAGAGATCAAGTCGATGTCAAAGATTGTGCC AACCATGAAATCAAAGAAGTTTTGG- 3'
	anti-sense	OB28	39-mer	5' -CGT*Xba I*CTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
H30N	sense	OB46	54-mer	5' -CGG*Kpn I*GGTACCAGGATGTCATGGTTCAGAACCATGTATCATTAA CCGTGGTAAACC- 3'
	anti-sense	OB28	39-mer	5' -CGT*Xba I*CTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
E62S	sense	OB47	33-mer	5' -GCCTCAATCGATGGTTTATCAGTTGATGTTCCC- 3'
	anti-sense	OB48	33-mer	5' -GGGAACATCAACTGATAAACCATCGATTGAGGC- 3'
H74N	sense	OB49	32-mer	5' -CATGG*Sph I*CATGCAATTACATGAAATGCCCATTGG- 3'
	anti-sense	OB28	39-mer	5' -CGT*Xba I*CTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
K82N	sense	OB50	50-mer	5'-CTAC*Sph I*GCATGCCATTACATGAAATGCCCATTGGTTAATGGACAA CAATATG- 3'
	anti-sense	OB28	39-mer	5' -CGT*Xba I*CTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'

Figure 32 (Der p 2)



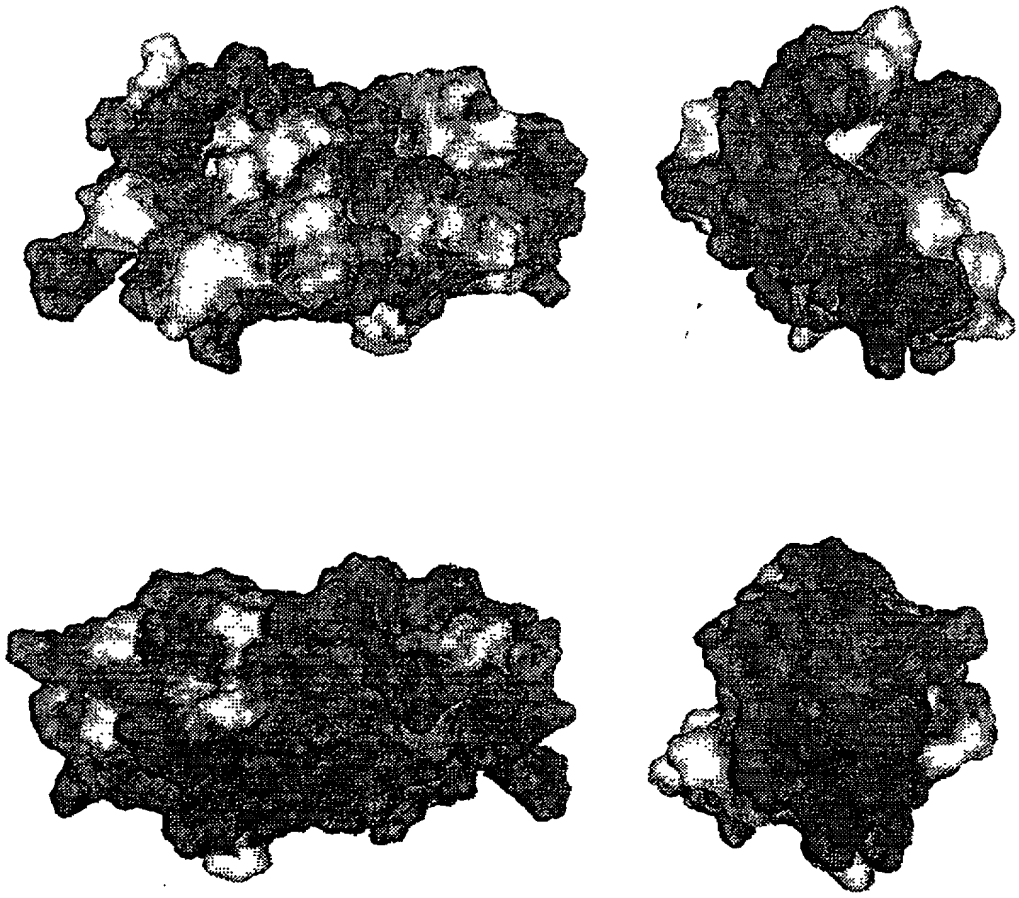


FIG. 33: Der p 2

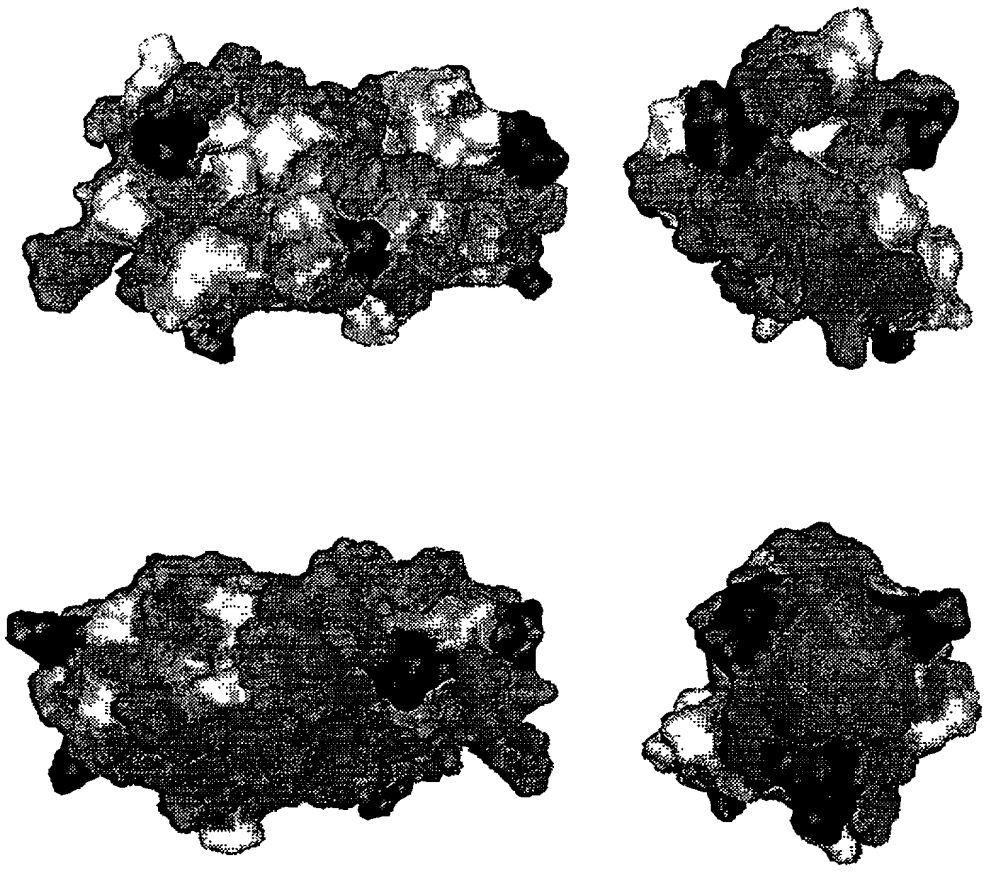


FIG. 34: Der p 2 mutant

Figure 35A (Der p 1)

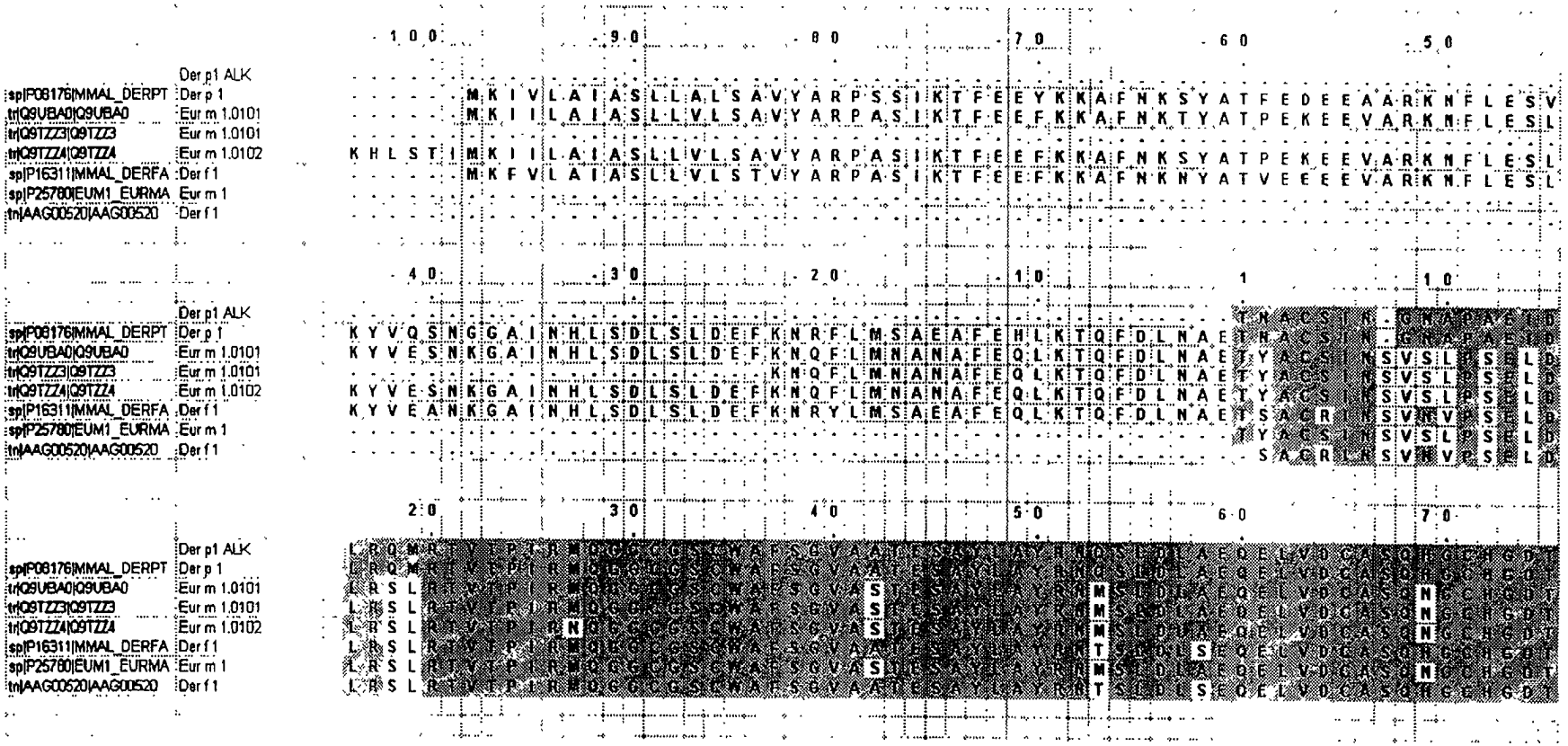
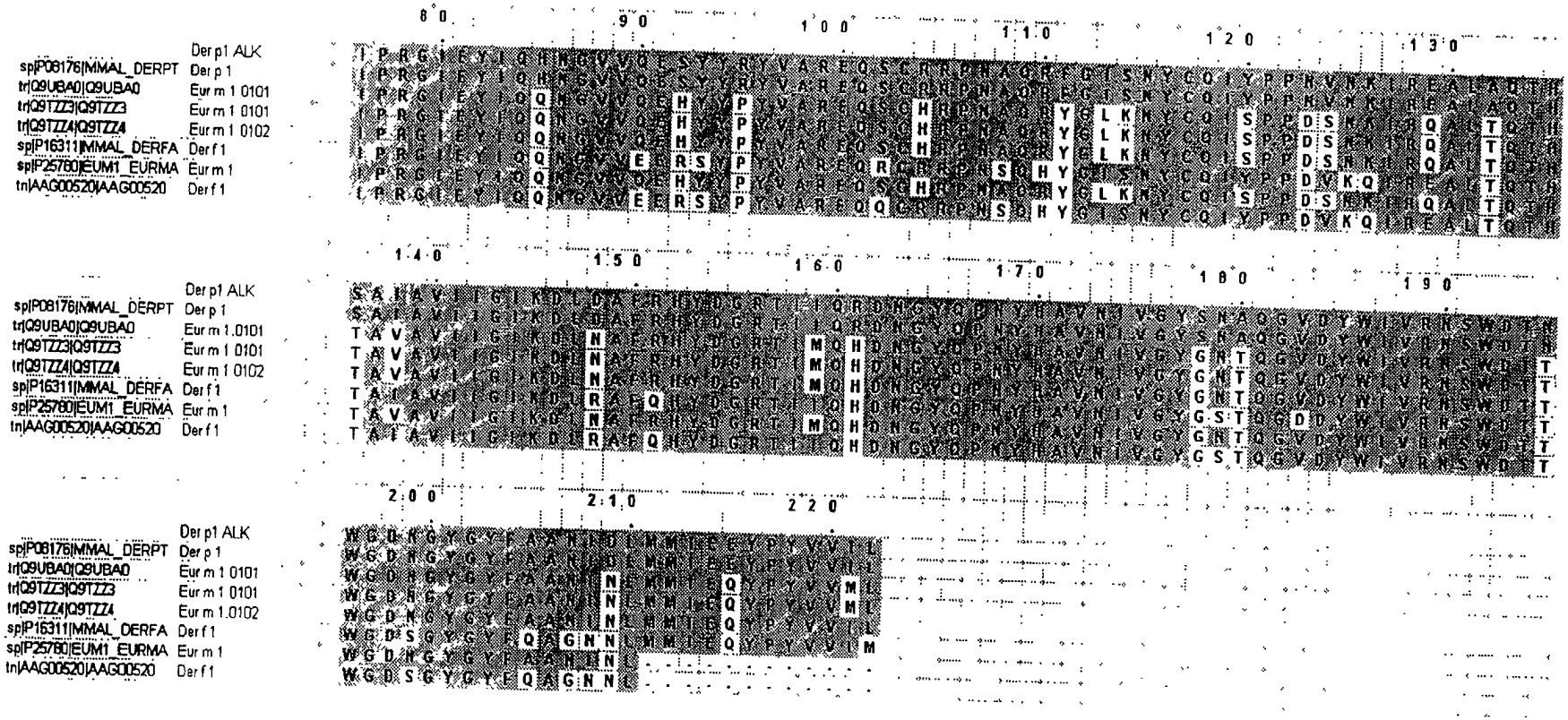


Figure 35B (Der p 1)



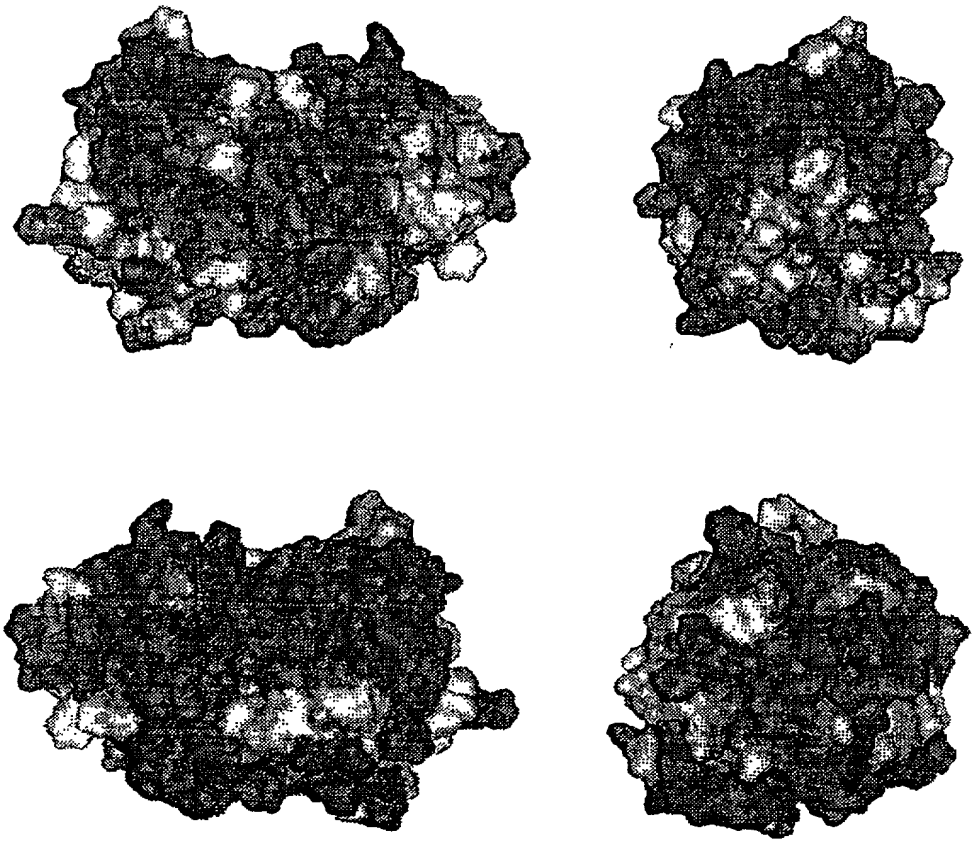


FIG. 36: Der p 1

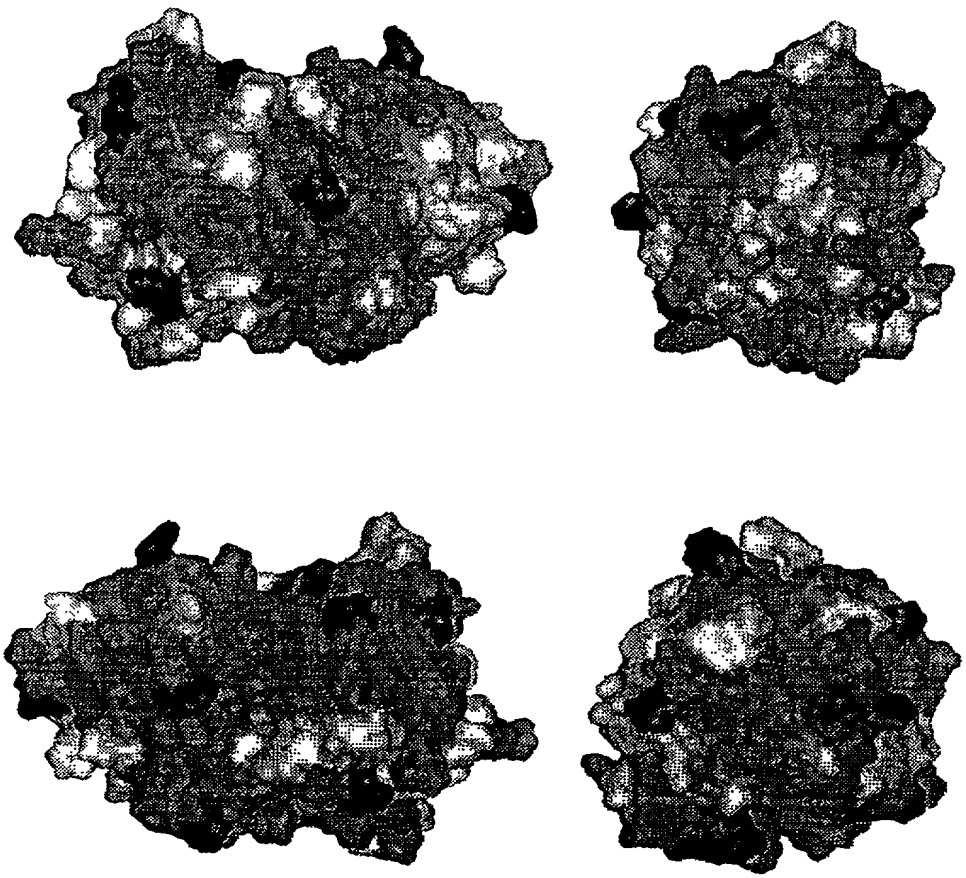


FIG. 37: Der p 1 mutant

FIG. 38A (Phl p 5)

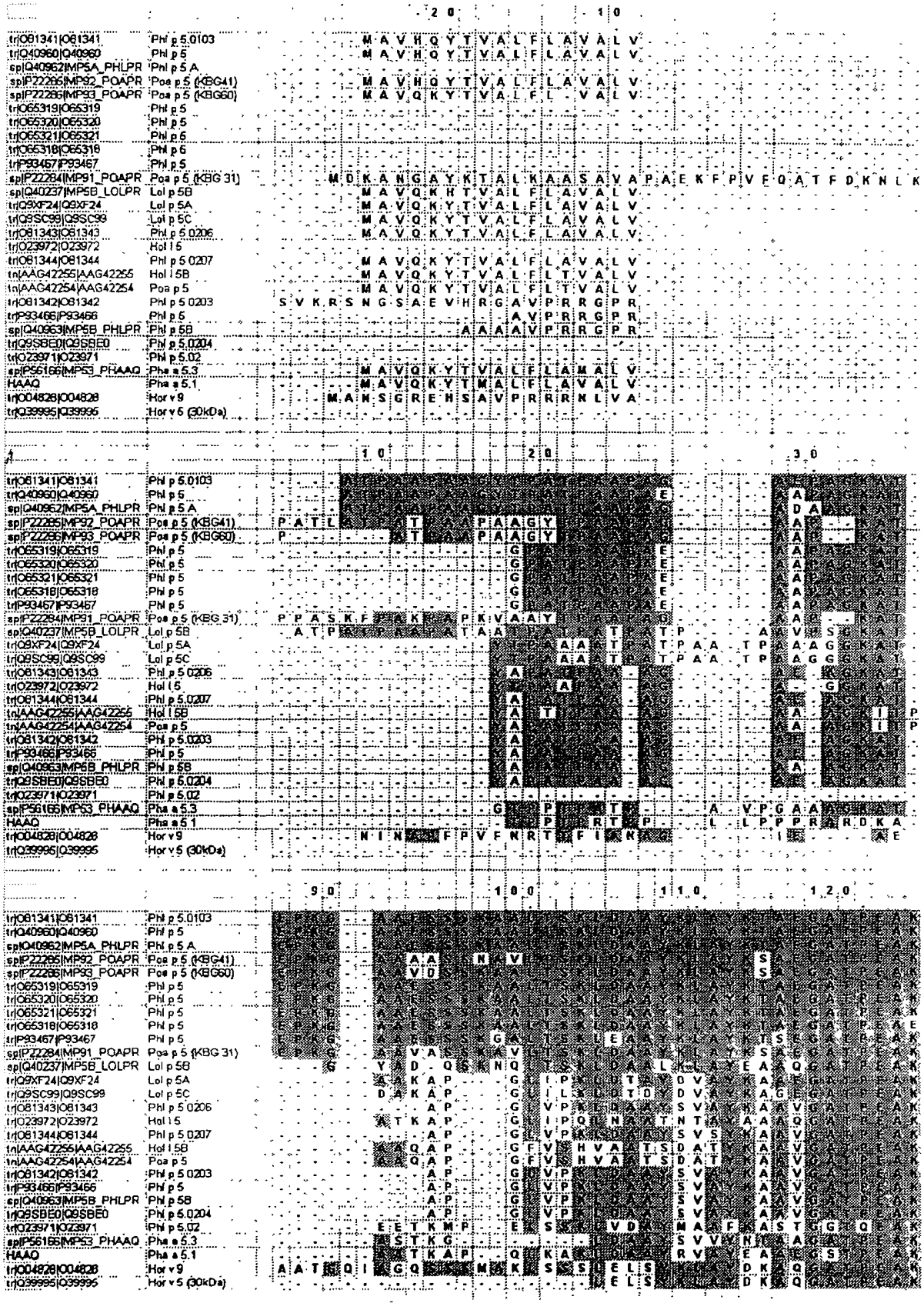


FIG. 38B (Phl p 5)

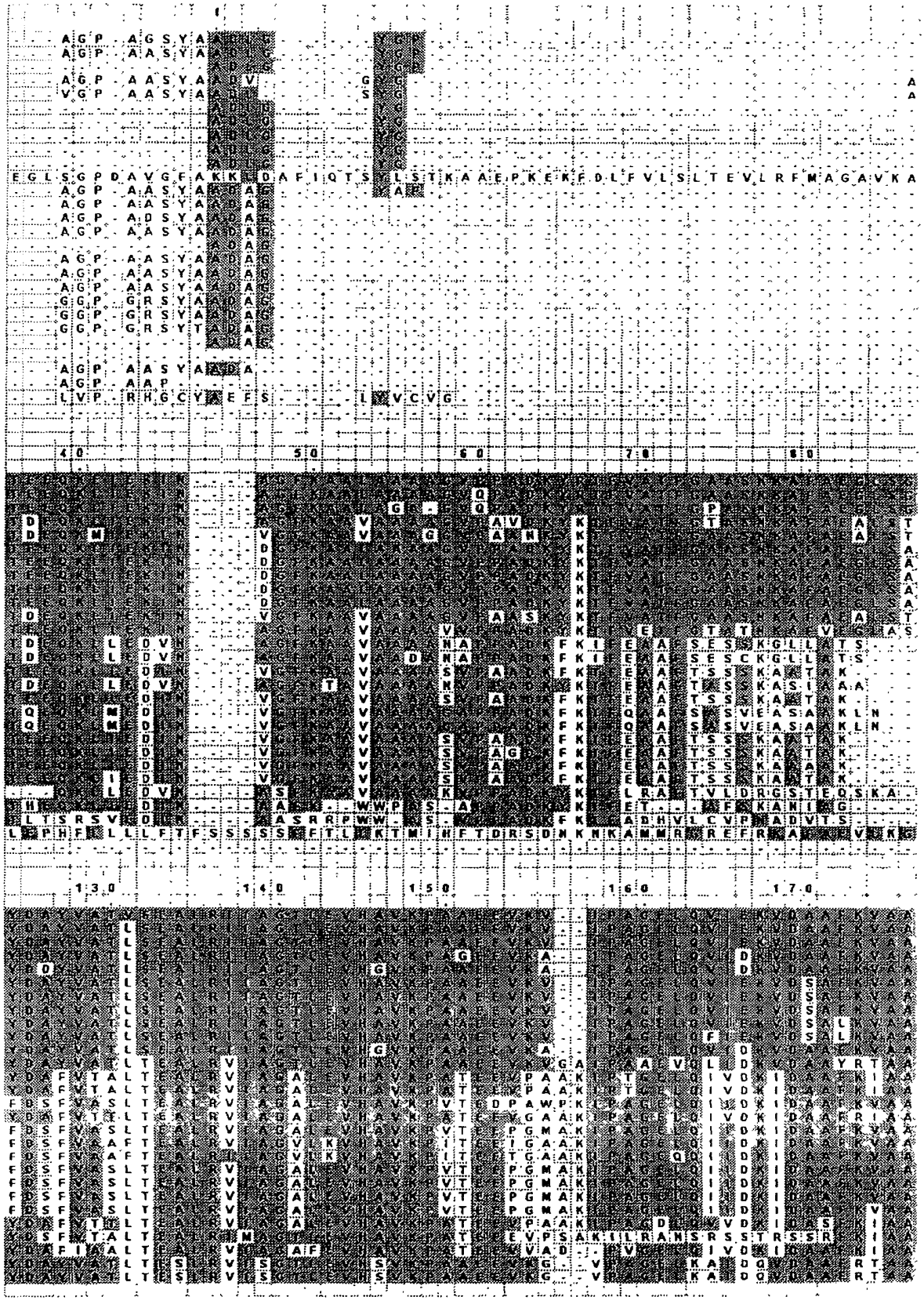


FIG. 38C (Phl p 5)

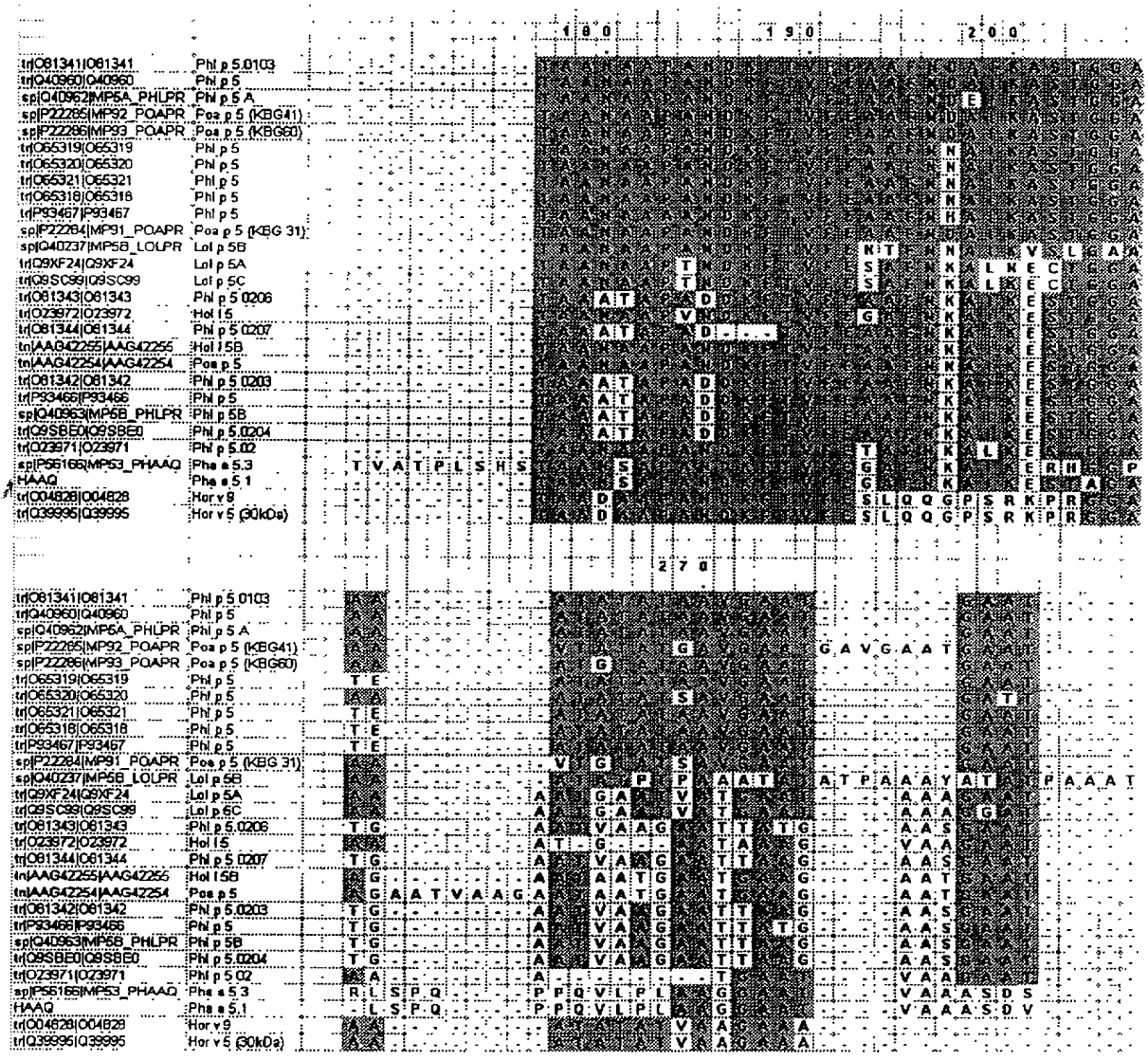
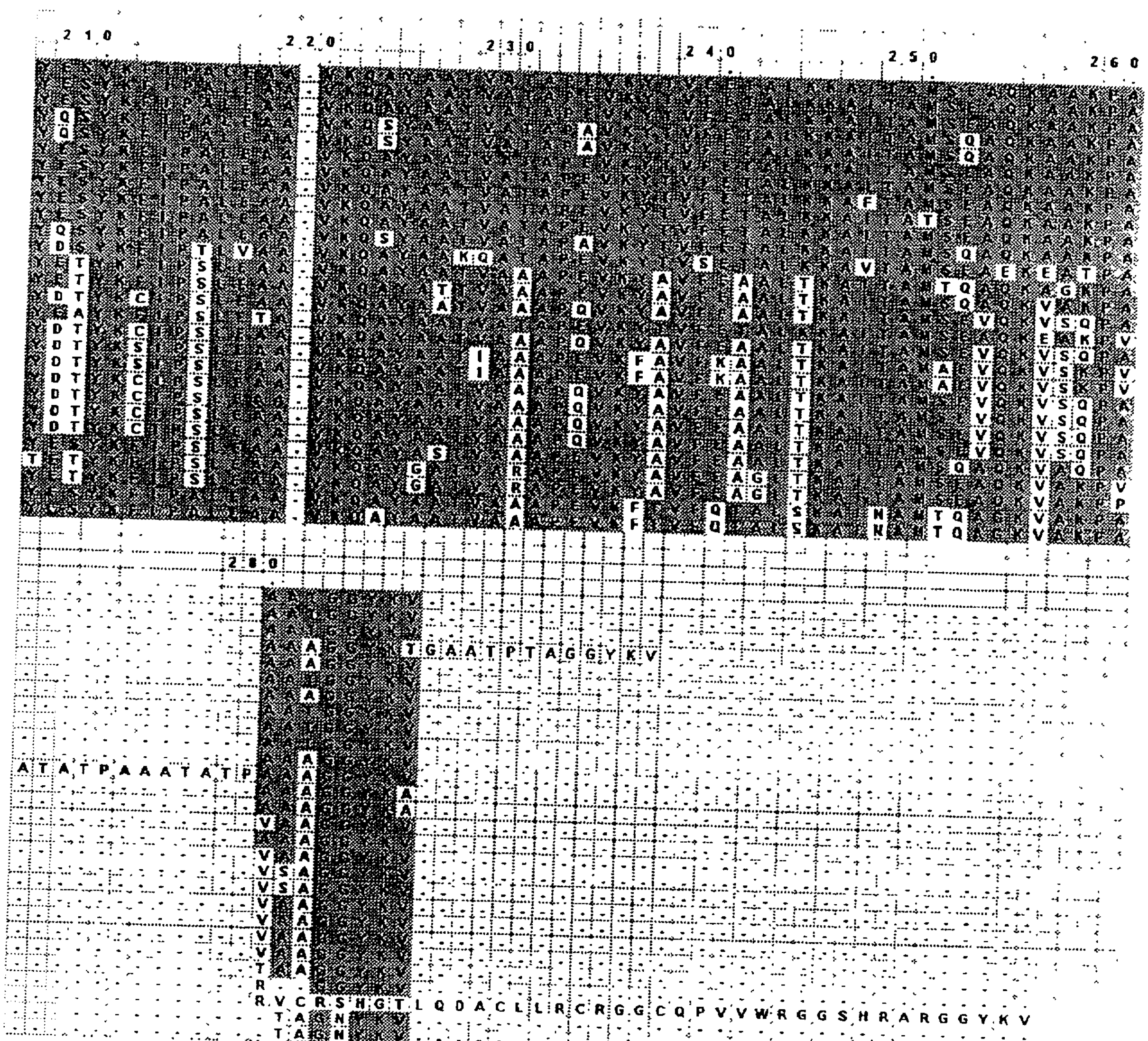


FIG. 38D (Phl p 5)



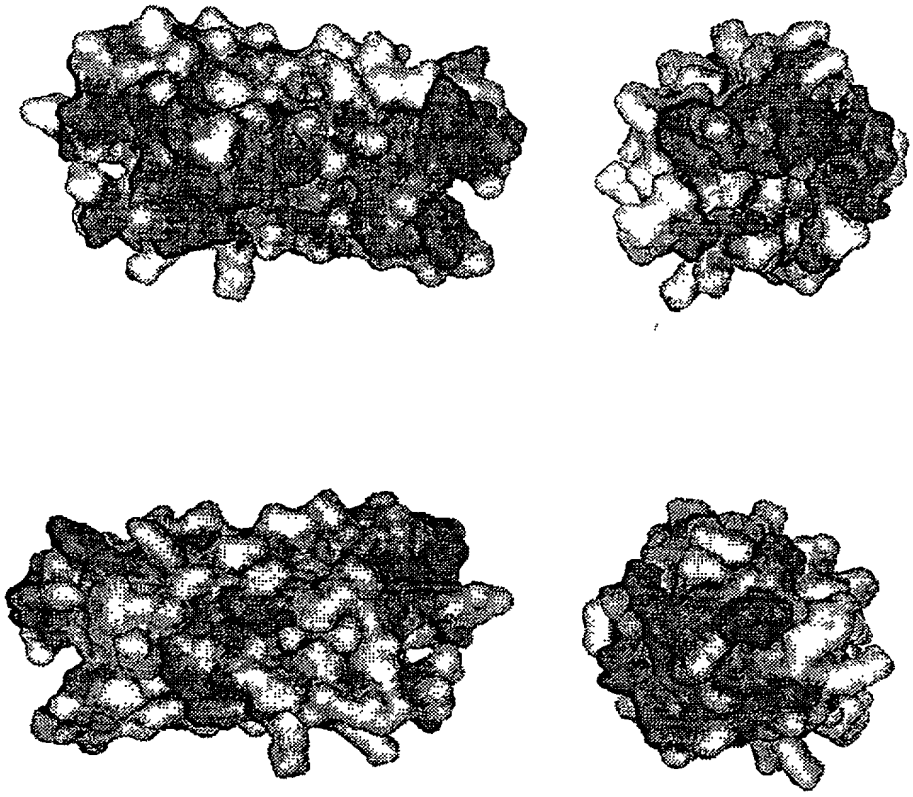


FIG. 39A: Phl p 5, Model A

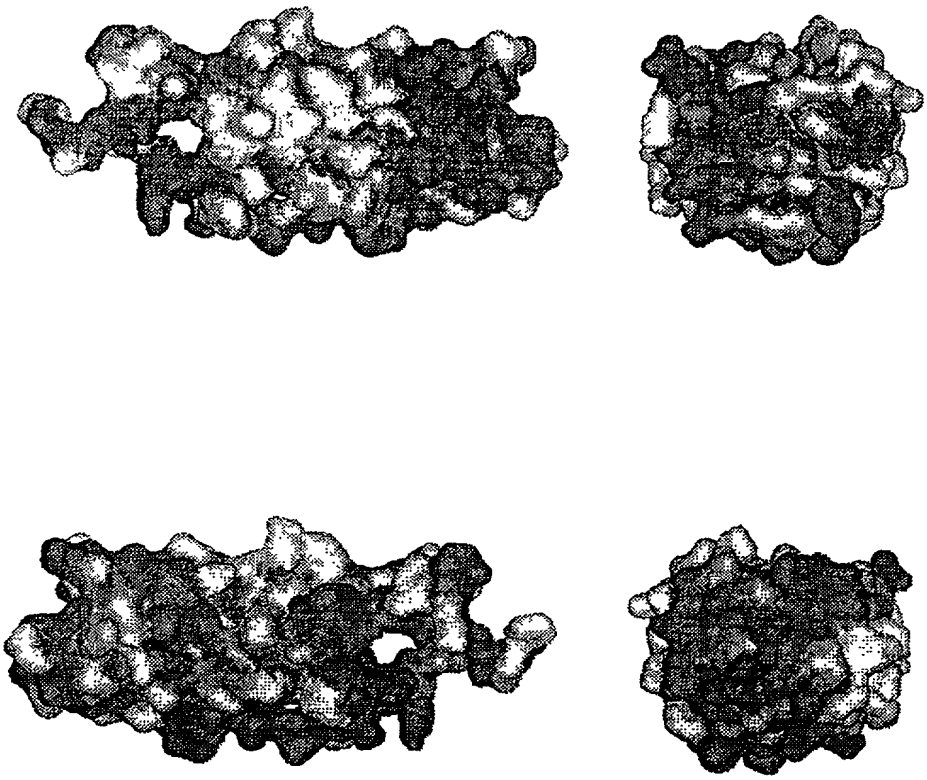


FIG. 39B: Phl p 5, Model B

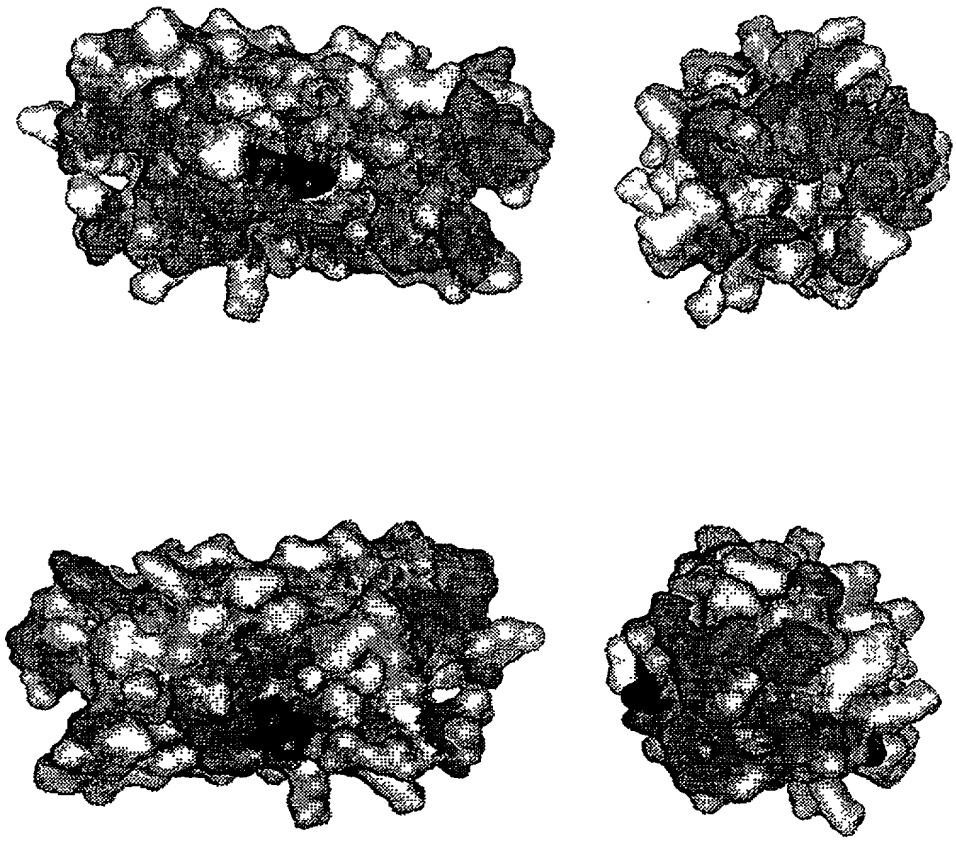


FIG. 40A: Phl p 5 mutant, Model A

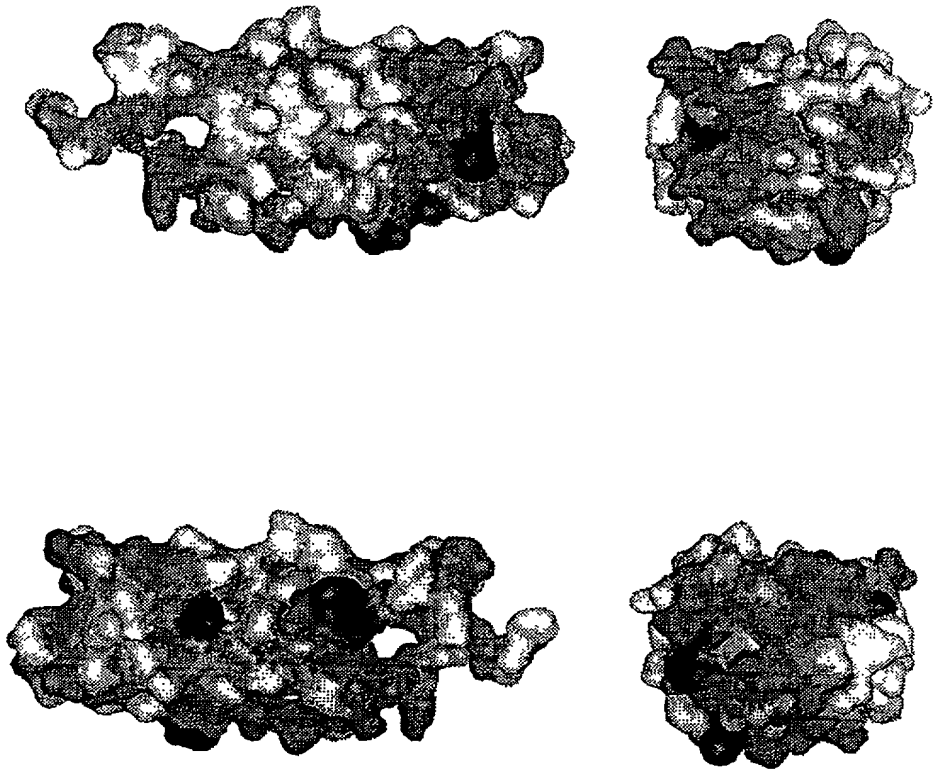


FIG. 40B: Phl p 5 mutant, Model B

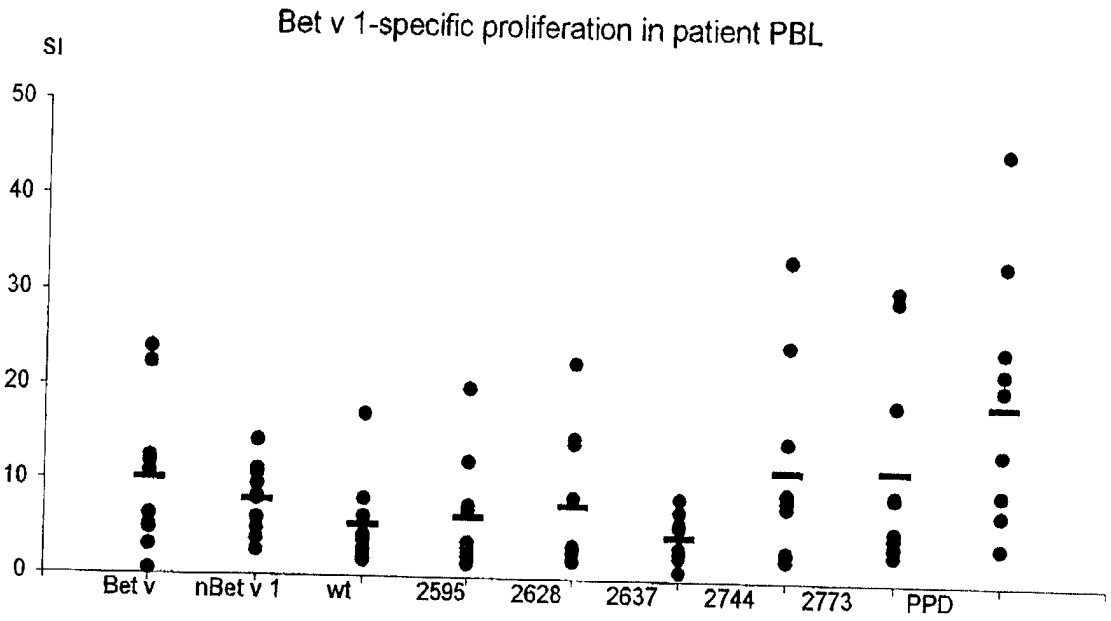


Figure 41: Stimulation of Bet v 1 samples

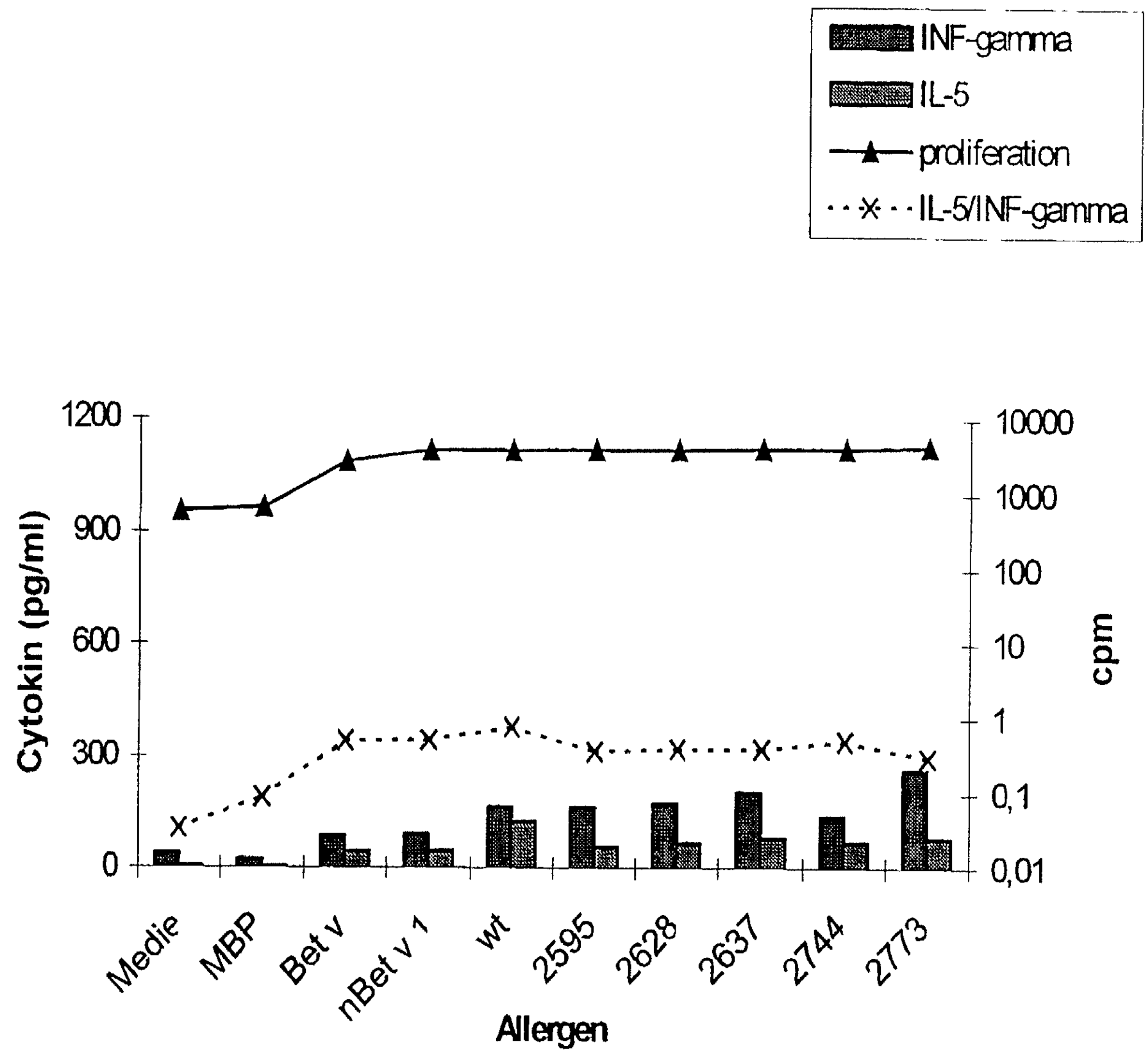

INF-gamma
IL-5
proliferation
IL-5/INF-gamma
Cytokin (pg/ml)
1200
900
600
300
0
cpm
10000
1000
100
10
1
0,1
0,01
Medie
MBP
Bet v
nBet v 1
wt
2595
2628
2637
2744
2773
Allergen

FIG. 42

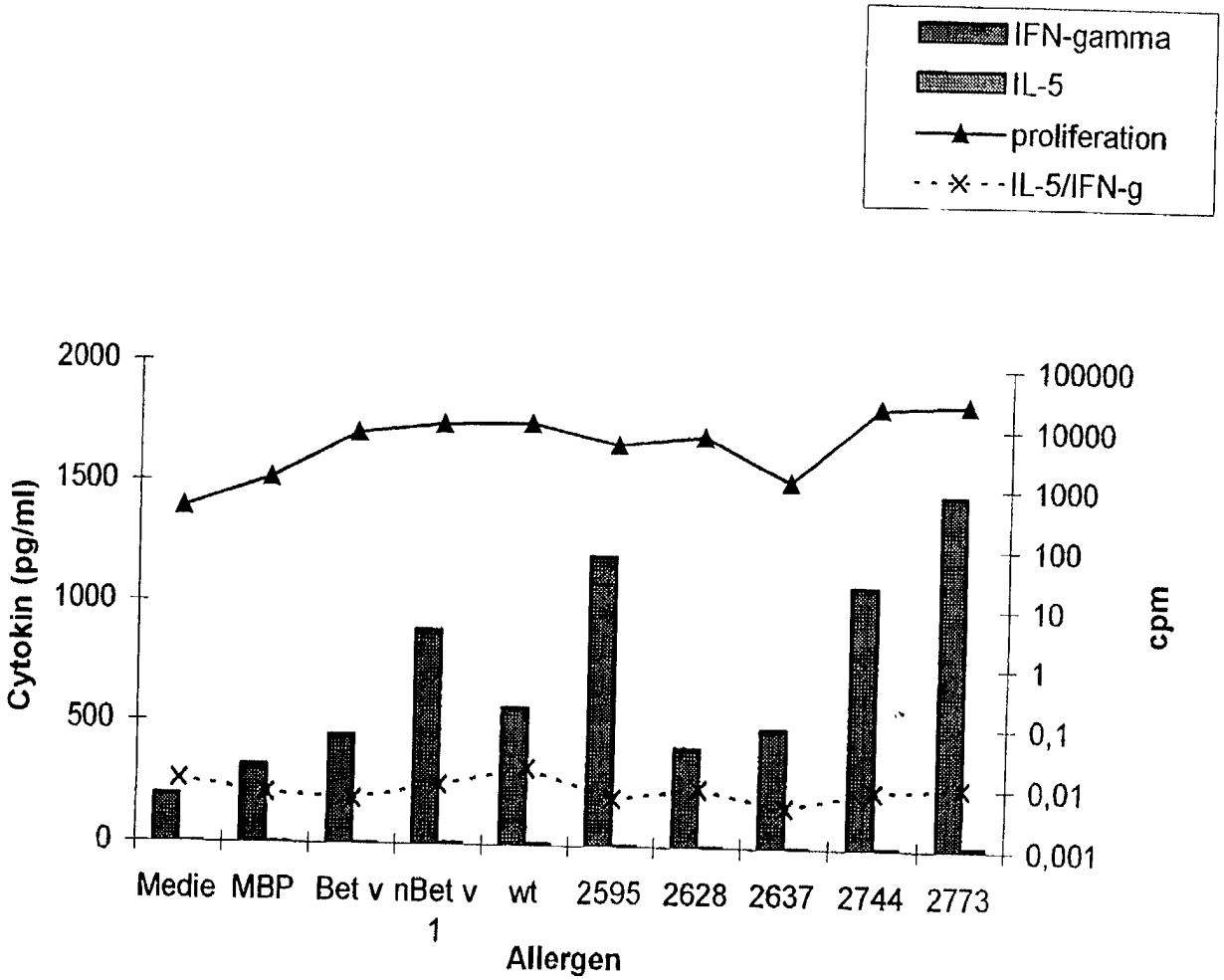


FIG. 43

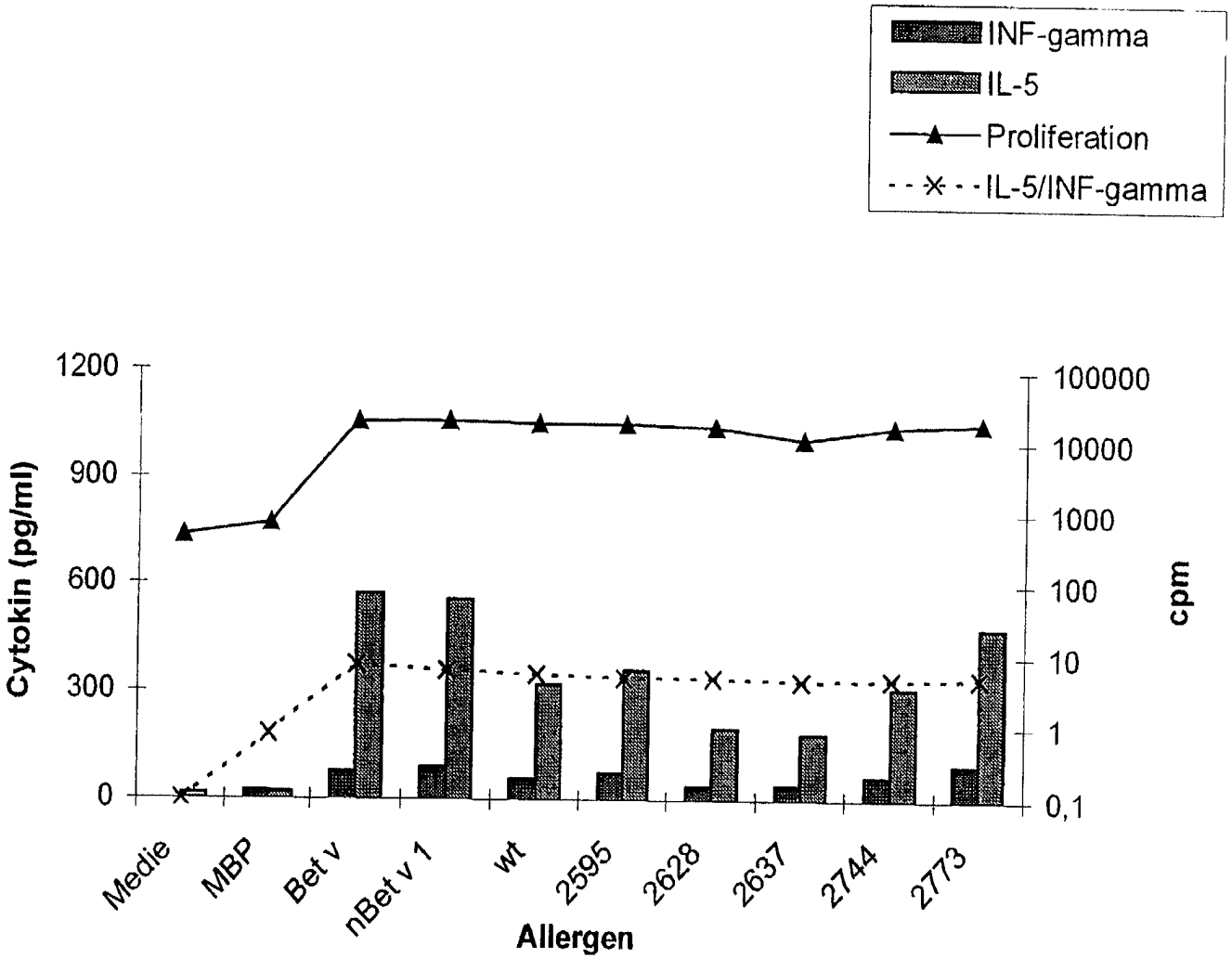


FIG. 44